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FILE 'HOME' ENTERED AT 17:12:24 ON 30 MAY 2001

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=> s bacteriorhodopsin

L1         6392 BACTERIORHODOPSIN

=> s l1 and (fusion or chimer?)

L2          116 L1 AND (FUSION OR CHIMER?)

=> dup rem l2

PROCESSING COMPLETED FOR L2
L3           63 DUP REM L2 (53 DUPLICATES REMOVED)

=> s l3 and py <1999

   2 FILES SEARCHED...
L4           49 L3 AND PY <1999

=> s l1 and fused

L5           52 L1 AND FUSED

=> s l5 not l2

L6           23 L5 NOT L2

=> dup rem l6

PROCESSING COMPLETED FOR L6
L7           12 DUP REM L6 (11 DUPLICATES REMOVED)

=> s l7 or l4

L8           61 L7 OR L4

=> d ibib abs 1-61


L8   ANSWER 1 OF 61  MEDLINE
ACCESSION NUMBER:    1999196667     MEDLINE
DOCUMENT NUMBER:     99196667   PubMed ID: 10094768
TITLE:               Extraction method for analysis of detergent-solubilized
                   bacteriorhodopsin and hydrophobic peptides by
                     electrospray ionization mass spectrometry.
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AUTHOR: Barr[redacted]ge D R; Dratz E A; Jesaitis A J; Sunner J
CORPORATE SOURCE: Dep[redacted]ment of Chemistry and Biochemis[redacted], Montana State University, 108 Gaines Hall, Bozeman, Montana 58717-3520, USA.
CONTRACT NUMBER: AI22735 (NIAID)
AI26711 (NIAID)
EY06913 (NEI)
SOURCE: ANALYTICAL BIOCHEMISTRY, (1999 Apr 10) 269 (1) 1-9.
Journal code: 4NK; 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199905
ENTRY DATE: Entered STN: 19990614
Last Updated on STN: 19990614
Entered Medline: 19990528

AB The analysis of integral membrane proteins or transmembrane peptides by electrospray ionization mass spectrometry (ESI-MS) is difficult since detergents, used to solubilize these hydrophobic proteins and peptides, severely suppress analyte ion formation. This problem has been addressed previously by precipitating the protein, removing the detergent, and resolubilizing the protein in a nonpolar solvent. Here, we demonstrate a method that avoids protein precipitation and resolubilization. Detergent-solubilized **bacteriorhodopsin** is extracted into a nonpolar solvent phase by adding a chloroform/methanol/water solvent mixture to the aqueous detergent solution. ESI mass spectra of the nonpolar, chloroform-rich phase were dominated by peaks due to bacterioopsin. Bacterioopsin precursors with partially cleaved leader sequences were seen in all mass spectra. Additional peaks were likely due to intact **bacteriorhodopsin**, i.e., bacterioopsin with the retinal prosthetic group attached, and to bacterioopsin associated with lipid molecules. A separation process that occurred in the **fused** -silica capillary leading to the electrospray tip was essential for obtaining ESI mass spectra of bacterioopsin. The extraction-into-chloroform procedure also worked well with hydrophobic, transmembrane-type peptides that were insoluble in other electrospray solvents, including 100% formic acid, and the method has application to transmembrane peptides formed from digests of integral membrane proteins.

L8 ANSWER 2 OF 61 MEDLINE
ACCESSION NUMBER: 1999026957 MEDLINE
DOCUMENT NUMBER: 99026957 PubMed ID: 9809429
TITLE: Functional expression of green fluorescent protein derivatives in Halobacterium salinarum.
AUTHOR: Nomura S; Harada Y
CORPORATE SOURCE: Biomolecular Engineering Research Institute, Osaka, Japan.
SOURCE: FEMS MICROBIOLOGY LETTERS, **(1998 Oct 15)** 167 (2) 287-93.
Journal code: FML; 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981214

AB We investigated the applicability of the green fluorescent protein (GFP) of Aequorea victoria as a reporter for gene expression in an extremely halophilic organism: Halobacterium salinarum. Two recombinant GFPs were fused with **bacteriorhodopsin**, a typical membrane protein of H. salinarum. These **fusion** proteins preserved the intrinsic functions of each component, **bacteriorhodopsin** and GFP, were expressed in H. salinarum under conditions with an extremely high salt

concentration, and were proved to be properly localized in its plasma membrane. These results suggest that GFP could be used as a versatile reporter of gene expression in H. salinarum for investigations of various halophilic membrane proteins, such as sensory rhodopsin or phoborhodopsin.

L8 ANSWER 3 OF 61 MEDLINE
ACCESSION NUMBER: 1998443247 MEDLINE
DOCUMENT NUMBER: 98443247 PubMed ID: 9769218
TITLE: A novel three-dimensional crystal of **bacteriorhodopsin** obtained by successive **fusion** of the vesicular assemblies.
AUTHOR: Takeda K; Sato H; Hino T; Kono M; Fukuda K; Sakurai I; Okada T; Kouyama T
CORPORATE SOURCE: Graduate School of Science, Nagoya University, Nagoya, 464-8602, Japan.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, **(1998 Oct 23)** 283 (2) 463-74.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981125

AB When the two-dimensional crystal of **bacteriorhodopsin** (bR), purple membrane, is incubated at high temperature (32 degreesC) with a small amount of the neutral detergent octylthioglucoside in the presence of the precipitant ammonium sulfate, a large fraction of the membrane fragments is converted into spherical vesicles with a diameter of 50 nm, which are able to assemble into optically isotropic hexagonal crystals when the precipitant concentration is increased. The vesicularization of purple membrane takes place under such a condition that the miscibility of the detergent to the aqueous phase becomes very low, and we suggest that it is initiated by insertion of the detergent molecules into the membrane.
At low temperature, the transformation into the vesicular structure is inhibited and no large crystal is produced directly from membrane/detergent/precipitant mixtures. When a suspension of the spherical vesicles produced at the high temperature is cooled and concentrated below 15 degreesC, however, a birefringent hexagonal crystal is produced that diffracts X-rays beyond 2.5 A resolution. This new crystal belongs to the space group P622 with unit cell dimensions of a=b=104.7 A and c=114.1 A, and it is shown to be made up of stacked planar membranes, in each of which the bR trimers are arranged on a honeycomb lattice and the space among the proteins is filled with the detergent molecules and native lipids. These stacked membranes are suggested to be produced by successive **fusion** of the spherical vesicles. This implies that the crystallization is achieved without any step for complete solubilization of the protein. The present result offers a unique crystallization method that may be applicable to such membrane proteins that are liable to denature in the presence of an excess amount of detergent.

L8 ANSWER 4 OF 61 MEDLINE
ACCESSION NUMBER: 1998111010 MEDLINE
DOCUMENT NUMBER: 98111010 PubMed ID: 9450549
TITLE: Functional expression of pharaonis phoborhodopsin in Escherichia coli.
AUTHOR: Shimono K; Iwamoto M; Sumi M; Kamo N
CORPORATE SOURCE: Laboratory of Biophysical Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan.

SOURCE: FEBS LETTERS, **(1997 Dec 22)** 420 (1) 5[illegible]6.
Journal code: EUH; 0155157. ISSN: 001[illegible]793.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980226
Last Updated on STN: 19980226
Entered Medline: 19980213

AB Pharaonis phoborhodopsin, the photoreceptor of the negative phototaxis of archaebacterial Natronobacterium pharaonis, was functionally expressed in the heterologous system of Escherichia coli. Flash-photolysis on a millisecond time scale indicated that the photochemical properties of ppR expressed in E. coli were the same as those of the native ppR in N. pharaonis. We concluded that the integral membrane protein ppR is correctly folded in vivo in the eubacterial E. coli membrane.

L8 ANSWER 5 OF 61 MEDLINE
ACCESSION NUMBER: 1998039537 MEDLINE
DOCUMENT NUMBER: 98039537 PubMed ID: 9372268
TITLE: Hydrophobic peptide mapping of clinically relevant heptathelical membrane proteins by capillary electrophoresis.
AUTHOR: Dong M; Oda R P; Strausbauch M A; Wettstein P J; Landers J P; Miller L J
CORPORATE SOURCE: Center for Basic Research in Digestive Diseases, Rochester,
MN 55905, USA.
CONTRACT NUMBER: DK 32878 (NIDDK)
DK 46577 (NIDDK)
SOURCE: ELECTROPHORESIS, (1997 Sep) 18 (10) 1767-74.
Journal code: ELE; 8204476. ISSN: 0173-0835.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980224
Last Updated on STN: 19980224
Entered Medline: 19980206

AB The structural investigation of G protein-coupled receptors has been hindered by the lack of techniques to effectively resolve the hydrophobic peptides obtained by chemical or proteolytic cleavage, as well as the minute amounts of protein typically isolated. We have developed a capillary electrophoresis method for efficient separation of hydrophobic peptides using a cyanogen bromide digest of **bacteriorhodopsin** as a model for these clinically important membrane proteins. This procedure includes (i) solubilization of the protein digest in acetic acid; and (ii) electrophoresis using an acetic acid-based buffer system augmented by acetonitrile and hexane sulfonic acid, in a Polybrene-coated **fused** silica capillary. The potential for detection sensitivity to be increased at least 100-fold by use of on-line solid-phase extraction on C18-silica is shown. This approach is potentially useful for peptide fingerprinting of sparse and extremely hydrophobic membrane receptors.

L8 ANSWER 6 OF 61 MEDLINE
ACCESSION NUMBER: 97348063 MEDLINE
DOCUMENT NUMBER: 97348063 PubMed ID: 9204130
TITLE: Molecular handling of photosynthetic proteins for molecular
assembly construction.
AUTHOR: Miyake J; Hara M
CORPORATE SOURCE: National Institute for Advanced Interdisciplinary Research,
AIST/MITI, Ibaraki, Japan.
SOURCE: ADVANCES IN BIOPHYSICS, **(1997)** 34 109-26. Ref: 102

Journal code: 2J2; 0262476. ISSN: 006[redacted]27X.
PUB. COUNTRY: Japa[redacted]
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970813
Last Updated on STN: 19970813
Entered Medline: 19970806

AB Methods of constructing proteins were examined with special reference to the molecular assembly using photosynthetic RCs as membrane proteins. Molecular assemblies at the interfaces were studied by LB films, adsorption to the surface and reconstitution into liposomes and bilayer lipid membranes. The applications of biological specific ligands (recognition and binding), combinatorial chemical method, 2-D and 3-D order array assemblies and modification of protein molecules to make **fusion** proteins, as well as physical methods of manipulation of molecules by AFM tips and electric fields were reviewed.

L8 ANSWER 7 OF 61 MEDLINE
ACCESSION NUMBER: 97202487 MEDLINE
DOCUMENT NUMBER: 97202487 PubMed ID: 9050006
TITLE: Expression of beta 2-adrenoceptors in halobacteria.
AUTHOR: Sohlemann P; Soppa J; Oesterhelt D; Lohse M J
CORPORATE SOURCE: Institute for Pharmacology and Toxicology, University of Wurzburg, Germany.
SOURCE: NAUNYN-SCHMIEDEBERGS ARCHIVES OF PHARMACOLOGY, **(1997 Feb)** 355 (2) 150-60.
Journal code: NTQ; 0326264. ISSN: 0028-1298.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970609
Last Updated on STN: 19970609
Entered Medline: 19970527

AB Halobacteria are halophilic representatives of the recently defined domain, the Archaea. Halobacterium salinarium belongs to this group of microorganisms and contains large amounts of **bacteriorhodopsin** in its membrane. **Bacteriorhodopsin** is a seven-transmembrane protein that consists of bacterio-opsin (BO), and the chromophore retinal, which is covalently attached to BO. We have investigated whether the expression machinery for BO can be utilized for synthesis of the human beta 2-adrenoceptor (beta 2-AR), a protein with a similar seven-transmembrane-helix topology. An expression vector for BO synthesis was modified to express beta 2-ARs under the control of BO regulatory clements in H. salinarium. Homologous recombination into the genome was verified by polymerase chain reactions. Northern blots revealed transcripts of the calculated size and significant amounts of epitope-tagged beta 2-ARs were detected in Western blots. However, binding of the beta-AR antagonist 125I-cyanopindolol revealed low levels of functional receptors, and the ligand binding properties of these receptors were altered when compared to native receptors. Expression of **chimeras** containing larger amino terminal portions of BO did not result in higher receptor levels. Expression of beta 2-AR in Haloferax volcanii, another member of halobacteria, was achieved with a vector carrying the ferredoxin promoter. The levels of functional receptor as determined by 125I-cyanopindolol binding were 180 fmol/mg protein. The beta-AR ligands isoprenaline and propranolol showed affinities expected for functional beta 2-ARs. Thus, functional human beta 2-ARs were expressed in halobacteria, constituting a first approach for expression of a eukaryotic protein in the domain of Archaea.

L8 ANSWER 8 OF 61 MEDLINE
ACCESSION NUMBER: 97086614 MEDLINE
DOCUMENT NUMBER: 97086614 PubMed ID: 8932303
TITLE: Deletion mapping of the sites on the HtrI transducer for sensory rhodopsin I interaction.
AUTHOR: Perazzona B; Spudich E N; Spudich J L
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, University of Texas-Houston Health Science Center 77030, USA.
CONTRACT NUMBER: R01-GM27750 (NIGMS)
SOURCE: JOURNAL OF BACTERIOLOGY, **(1996 Nov)** 178 (22) 6475-8.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19970107

AB The phototaxis receptor sensory rhodopsin I (SRI) transmits signals through a membrane-bound transducer protein, HtrI. The genes for the receptor and transducer, sopI and htrI, respectively, are normally cotranscribed; however, previous work has established that fully functional interacting proteins are produced when htrI is expressed from the chromosome and sopI is expressed from a different promoter on a plasmid. In this report we show that in the membrane, concentrations of SRI from plasmid expression of wild-type sopI are negligible in the absence of HtrI protein in the cell. This requirement for HtrI is eliminated when sopI is extended at the 5'-end with 63 nucleotides of the bop gene, which encodes the N-terminal signal sequence of the **bacteriorhodopsin** protein. The signal is cleaved from the **chimeric** protein, and processed SRI is stable in the HtrI-free membrane. These results suggest a chaperone-like function for HtrI that facilitates membrane insertion or proper folding of the SRI protein. Six deletion constructs of HtrI were examined to localize the interaction sites for its putative chaperone function and for HtrI control of the SRI photocycle, a phenomenon described previously. The smallest HtrI fragment identified, which contained interaction sites for both SRI stability and photocycle control, consisted of the N-terminal 147 residues of the 536-residue HtrI protein. The active fragment is predicted to contain two transmembrane helices and the first approximately 20% of the cytoplasmic portion of the protein.

L8 ANSWER 9 OF 61 MEDLINE
ACCESSION NUMBER: 97022122 MEDLINE
DOCUMENT NUMBER: 97022122 PubMed ID: 8868482
TITLE: Overexpression of bacterio-opsin in Escherichia coli as a water-soluble **fusion** to maltose binding protein: efficient regeneration of the **fusion** protein and selective cleavage with trypsin.
AUTHOR: Chen G Q; Gouaux J E
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University of Chicago, Illinois 60637, USA.
SOURCE: PROTEIN SCIENCE, **(1996 Mar)** 5 (3) 456-67.
Journal code: BNW; 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970313
Last Updated on STN: 19970313
Entered Medline: 19970304

AB **Bacteriorhodopsin** (bR) is a light-driven proton pump from Halobacterium salinarium and is a model system for studying membrane protein folding, stability, function, and structure. bR is composed of

bacterio-opsin (bO), the 248-amino acid apo protein, and all-trans retinal, which is linked to lysine 216 via a protonated Schiff base. A bO gene (sbOd) possessing 29 unique restriction sites and a carboxyl-terminal purification epitope (1D4, nine amino acids) has been designed and synthesized. Overexpression of bO was achieved by **fusion** to the carboxyl terminus of maltose binding protein (MBP). The expressed **fusion** protein (MBP-sbO-1D4) formed inclusion bodies in Escherichia coli and, following solubilization with urea and removal of the urea by dialysis, approximately 170 mg of approximately 75% pure MBP-sbO-1D4 was obtained from 1 L of culture. MBP-sbO-1D4 formed high molecular weight (> or = 2,000 kDa) oligomers that were water-soluble. The synthetic bO with the 1D4 tag (sbO-1D4) was separated from MBP by trypsin cleavage at the factor Xa site between the MBP and sbO-1D4 domains. Selective trypsin cleavage at the factor Xa site, instead of at the 14 other potential trypsin sites within bO, was accomplished by optimization of the digestion conditions. Both MBP-sbO-1D4 and sbO-1D4 were regenerated with all-trans retinal and purified to homogeneity. In general, 6-10 mg of sbR-1D4 and 52 mg of MBP-sbR-1D4 were obtained from 1 L of cell culture. No significant differences in terms of UV/vis light absorbance, light/dark adaptation, and photocycle properties were observed among sbR-1D4, MBP-sbR-1D4, and bR from H. salinarium.

L8 ANSWER 10 OF 61 MEDLINE

ACCESSION NUMBER: 96363464 MEDLINE
DOCUMENT NUMBER: 96363464 PubMed ID: 8746630
TITLE: Rapid high-yield purification and liposome reconstitution of polyhistidine-tagged sensory rhodopsin I.
AUTHOR: Krebs M P; Spudich E N; Spudich J L
CORPORATE SOURCE: Department of Microbiology & Molecular Genetics, University of Texas-Medical School Health Science Center, Houston 77030, USA.
CONTRACT NUMBER: R01 GM27750 (NIGMS)
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, **(1995 Dec)** 6 (6) 780-8.
Journal code: BJV; 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961210

AB We have used Ni(2+)-affinity chromatography as a rapid and efficient method to purify a sensory rhodopsin I (SR-I) derivative containing six consecutive histidine residues at its C-terminus (His-tagged SR-I). The protein was expressed in Halobacterium salinarium by integrating the corresponding gene at the chromosomal bacterioopsin locus under the control of the bacterioopsin promoter. His-tagged SR-I retains native SR-I photochemical reactions in purified membranes and phototaxis signaling function in vivo. Immobilized Ni(2+)-affinity chromatography of membranes solubilized in 1% layryl maltoside provides a single-step purification of the protein to electrophoretic homogeneity (> or = 90% pure). The procedure yields 1.7 mg pure photoactive protein/liter of culture (60% efficiency). This yield combined with engineered overproduction of the protein provides at least 120-fold greater amounts than that of a previously reported multistep purification procedure, permitting structural and biochemical analysis previously not feasible. The purified protein in lauryl maltoside at pH 5.3 exhibits a visible absorption maximum at 587 nm characteristic of SR-I. Spectrometric titration reveals an alkaline-induced species at 550 nm previously observed with transducer-free SR-I in native membranes. A previously unreported

structured absorpt band at 400 nm, consistent wi a deprotonated Schiff base, forms ith the same pKa as the 550-nm species. His-tagged SR-I reconstituted into phosphatidylglycerol proteoliposomes retains properties of transducer-free SR-I in native membranes: its flash-induced absorption difference spectrum is identical, its photochemical reaction cycle kinetics show a similar pH dependence, and it forms a photoactive 550-nm species under alkaline conditions. These results indicate His-tagged SR-I reconstituted in proteoliposomes is suitable for analyzing SR-I interaction with its transducer protein in vitro.

L8 ANSWER 11 OF 61 MEDLINE
ACCESSION NUMBER: 96293447 MEDLINE
DOCUMENT NUMBER: 96293447 PubMed ID: 8692917
TITLE: Mutational analysis and molecular modeling of the nonapeptide hormone binding domains of the [Arg8]vasotocin receptor.
AUTHOR: Hausmann H; Richters A; Kreienkamp H J; Meyerhof W; Mattes H; Lederis K; Zwiers H; Richter D
CORPORATE SOURCE: Institut fur Zellbiochemie und klinische Neurobiologie, Universitat Hamburg, Germany.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1996 Jul 9)** 93 (14) 6907-12.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960911
Last Updated on STN: 19970203
Entered Medline: 19960829

AB To identify determinants that form nonapeptide hormone binding domains of the white sucker Catostomus commersoni [Arg8]vasotocin receptor, **chimeric** constructs encoding parts of the vasotocin receptor and parts of the isotocin receptor have been analyzed by [(3,5-3H)Tyr2, Arg8]vasotocin binding to membranes of human embryonic kidney cells previously transfected with the different cDNA constructs and by functional expression studies in Xenopus laevis oocytes injected with mutant cRNAs. The results indicate that the N terminus and a region spanning the second extracellular loop and its flanking transmembrane segments, which contains a number of amino acid residues that are conserved throughout the nonapeptide receptor family, contribute to the affinity of the receptor for its ligand. Nonapeptide selectivity, however, is mainly defined by transmembrane region VI and the third extracellular loop. These results are complemented by a molecular model of the vasotocin receptor obtained by aligning its sequence with those of other G-protein coupled receptors as well as that of **bacteriorhodopsin**. The model indicates that amino acid residues of transmembrane regions II-VII that are located close to the extracellular surface also contribute to the binding of vasotocin.

L8 ANSWER 12 OF 61 MEDLINE
ACCESSION NUMBER: 96078828 MEDLINE
DOCUMENT NUMBER: 96078828 PubMed ID: 7579649
TITLE: Engineering membrane proteins.
AUTHOR: Popot J L; Saraste M
CORPORATE SOURCE: Institut de Biologie Physico-Chimique, Paris, France.
SOURCE: CURRENT OPINION IN BIOTECHNOLOGY, **(1995 Aug)** 6 (4) 394-402. Ref: 90
Journal code: A92; 9100492. ISSN: 0958-1669.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)

LANGUAGE: Eng[redacted]
FILE SEGMENT: Pri[redacted]ty Journals
ENTRY MONTH: 199512
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 20000303
Entered Medline: 19951207

AB Much of the research on integral membrane proteins mirrors that on soluble proteins; however, membrane protein engineering also has its own ends and means, many of which take advantage of the peculiar situation of membrane proteins, whose chains are distributed between one lipidic and two aqueous phases. Extramembrane loops have been shortened, cut, or elongated with segments forming proteolytic cleavage sites, foreign epitopes, extra transmembrane segments, or even whole proteins, with the aim of facilitating purification, biochemical/biophysical studies, or crystallogenesis. Transmembrane alpha-helices have been deleted, duplicated, exchanged, transported into a foreign context or replaced with synthetic peptides, in order to both understand their integration into, and assembly in, the membrane and unravel their functional role. Insertion of cysteine residues has been the basis for a great diversity of experiments, ranging from the exploration of secondary, tertiary and quaternary structures of the transmembrane region to the creation of anchoring points for reporter molecules. Chemical engineering--the synthesis of protein fragments or even of whole proteins--offers particularly exciting new prospects, given the small size of folding domains in alpha-helical membrane proteins. Membrane protein engineering is rapidly developing its own agenda of questions and tool chest of techniques.

L8 ANSWER 13 OF 61 MEDLINE
ACCESSION NUMBER: 95286981 MEDLINE
DOCUMENT NUMBER: 95286981 PubMed ID: 7769231
TITLE: Use of antibody fragments (Fv) in immunocytochemistry.
AUTHOR: Kleymann G; Ostermeier C; Heitmann K; Haase W; Michel H
CORPORATE SOURCE: Max-Planck-Institut Fur Biophysik, Abteilung Molekulare Membranbiologie, Frankfurt, Germany.
SOURCE: JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY, (1995 Jun) 43 (6) 607-14.
Journal code: IDZ; 9815334. ISSN: 0022-1554.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950713
Last Updated on STN: 19950713
Entered Medline: 19950706

AB We developed a novel antibody fragment (Fv) technique for localization and determination of the surface topology of membrane protein complexes by immunogold electron microscopy. Several hybridoma cell lines producing murine monoclonal antibodies (MAbs) raised against bacterial membrane proteins were established. The cDNAs coding for the variable domains of the MAbs were cloned and expressed in Escherichia coli. The engineered Fv fragments served as trifunctional adapter molecules. The Fv fragment binds to the epitope of the membrane protein. The Strep tag **fused** to the VH chain was used for one-step affinity purification of the Fv fragments. Immunological detection of the membrane protein-bound Fv fragments in electron microscopy was accomplished either via the Strep tag with colloidal gold-labeled streptavidin or via the c-myc tag, which was **fused** to the VL chain, in combination with the c-myc tag-specific antibody 9E10 and a colloidal gold-labeled secondary antibody. We examined four Fv fragments directed against the cytochrome c oxidase or the

ubiquinol-cytochrome c oxidoreductase of Paracoccus nitrificans and **bacteriorhodopsin** of Halobacterium halobium to show that this method is generally applicable. In all cases the Fv fragments showed the same results as their corresponding parent antibodies in electron microscopic immunostaining and other applications.

L8 ANSWER 14 OF 61 MEDLINE
ACCESSION NUMBER: 95023912 MEDLINE
DOCUMENT NUMBER: 95023912 PubMed ID: 7937771
TITLE: Photoactive mitochondria: in vivo transfer of a light-driven proton pump into the inner mitochondrial membrane of Schizosaccharomyces pombe.
AUTHOR: Hoffmann A; Hildebrandt V; Heberle J; Buldt G
CORPORATE SOURCE: Forschungszentrum Julich, Institut fur Biologische Informationsverarbeitung, Germany.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Sep 27) 91 (20) 9367-71. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19970203
Entered Medline: 19941027

AB The light-driven proton pump **bacteriorhodopsin** (bR) from Halobacterium salinarium has been genetically transferred into the inner mitochondrial membrane (IM) of the eukaryotic cell Schizosaccharomyces pombe, where the archaebacterial proton pump replaces or increases the proton gradient usually formed by the respiratory chain. For targeting and integration, as well as for the correct orientation of bR in the IM, the bacterioopsin gene (bop) was **fused** to signal sequences of IM proteins. Northern and Western blot analysis proved that all hybrid gene constructs containing the bop gene and a mitochondrial signal sequence were expressed and processed to mature bR. Fast transient absorption spectroscopy showed photocycle activity of bR integrated in the IM by formation of the M intermediate. Experiments with the pH-sensitive fluorescence dye 2',7'-bis(2-carboxyethyl)-5 (and -6)-carboxyfluorescein revealed bR-mediated proton pumping from the mitochondrial matrix into the intermembrane space. Glucose uptake measurements under anaerobic conditions showed that yeast cells containing photoactive mitochondria need less sugar under illumination. In summary, our experiments demonstrate the functional genetic transfer of a light energy converter to a naturally nonphotoactive eukaryotic organism.

L8 ANSWER 15 OF 61 MEDLINE
ACCESSION NUMBER: 95020638 MEDLINE
DOCUMENT NUMBER: 95020638 PubMed ID: 7934922
TITLE: A C-terminal truncation results in high-level expression of the functional photoreceptor sensory rhodopsin I in the archaeon Halobacterium salinarium.
AUTHOR: Ferrando-May E; Brustmann B; Oesterhelt D
CORPORATE SOURCE: Max-Planck-Institut fur Biochemie, Martinsried, Germany.
SOURCE: MOLECULAR MICROBIOLOGY, **(1993 Sep)** 9 (5) 943-53. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199411
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19941222
Entered Medline: 19941114

AB Expression of the gene encoding the halobacterial photoreceptor sensory

rhodopsin I (SRI), sopI, was studied by means of homologous gene targeting. A sopI- Halobacterium salinarium mutant strain was constructed by homologous replacement of sopI with a novobiocin-resistant gyrB from Haloferax Aa 2.2. Cells bearing gyrB were resistant to novobiocin, indicating that the Haloferax gene is functional in H. salinarium. Complementation of this deletion strain with sopI fused to the bacterio-opsin promoter resulted in the recovery of all phenotypical attributes of SRI. This establishes the first direct correlation between sopI and the function of its gene product. In the complemented deletion strain, functional expression of sopI occurred from the bop locus, where sopI had integrated by homologous recombination. This shows that cotranscription of sopI and the gene encoding the SRI signal transducer, htrI, which is found in the wild type, is not a prerequisite for photosensory activity. Deletion of the last 43 bp at the 3' end of sopI resulted in a 10-fold increase in the amount of SRI, without affecting the activity of the pigment. The mRNA level of the truncated gene was not affected as compared to that of the wild type. We propose that regulation occurs at the protein level, probably through a negative determinant of protein stability located in the C-terminus of SRI. Replacement of the last 28 amino acids of **bacteriorhodopsin** by the last 29 amino acids of SRI results in a decrease of the **bacteriorhodopsin**, supporting our observations. The C-terminus of SRI is the first domain with a downregulating influence on protein levels thus far identified in H. salinarium. The system for SRI overexpression we present here greatly facilitates biochemical and biophysical studies on the photoreceptor and allows investigation of the molecular interactions underlying the signal transduction chain of halobacterial phototaxis.

L8 ANSWER 16 OF 61 MEDLINE

ACCESSION NUMBER: 94316658 MEDLINE
DOCUMENT NUMBER: 94316658 PubMed ID: 8041764
TITLE: Tyr-129 is important to the peptide ligand affinity and selectivity of human endothelin type A receptor.
AUTHOR: Lee J A; Elliott J D; Sutiphong J A; Friesen W J; Ohlstein E H; Stadel J M; Gleason J G; Peishoff C E
CORPORATE SOURCE: Department of Macromolecular Sciences, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1994 Jul 19)** 91 (15) 7164-8.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940905
Last Updated on STN: 19940905
Entered Medline: 19940822

AB Molecular modeling and protein engineering techniques have been used to study residues within G-protein-coupled receptors that are potentially important to ligand binding and selectivity. In this study, Tyr-129 located in transmembrane domain 2 of the human endothelin (ET) type A receptor A (hETA) was targeted on the basis of differences between the hETA and type B receptor (hETB) sequences and the position of the residue on ET receptor models built using the coordinates of **bacteriorhodopsin**. Replacement of Tyr-129 of hETA by alanine, glutamine, asparagine, histidine, lysine, serine, or phenylalanine results in receptor variants with enhanced ET-3 and sarafotoxin 6C affinities but with unchanged ET-1 and ET-2 affinities. Except for Tyr-129-->Phe hETA, these hETA variants have two to three orders of magnitude lower binding affinity for the ETA-selective antagonist BQ123. Replacement of His-150, the residue in hETB that is analogous in sequence to Tyr-129 of hETA, by either tyrosine or alanine does not affect the affinity of peptide ligands. These results indicate that although transmembrane domain 2 is important in ligand selectivity for hETA, it does not play a significant role in the lack of ligand selectivity shown by hETB. **Chimeric**

receptors have been constructed that further support these conclusions and indicate that at least two hETA regions contribute to ligand selectivity. Additionally, the data support an overlap in the binding site in hETA of agonists ET-3 and sarafotoxin 6C with that of the antagonist BQ123.

L8 ANSWER 17 OF 61 MEDLINE
ACCESSION NUMBER: 94281539 MEDLINE
DOCUMENT NUMBER: 94281539 PubMed ID: 8011934
TITLE: Comparative studies on ion pumps of the bacterial rhodopsin family.
AUTHOR: Mukohata Y
CORPORATE SOURCE: Department of Biology, School of Science, Nagoya University, Japan.
SOURCE: BIOPHYSICAL CHEMISTRY, **(1994 May)** 50 (1-2) 191-201. Ref: 38
Journal code: A5T; 0403171. ISSN: 0301-4622.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 19940810
Last Updated on STN: 19940810
Entered Medline: 19940728

AB **Bacteriorhodopsin** (proton pump), halorhodopsin (anion pump), sensory rhodopsin and phoborhodopsin (photosensors) are found in Halobacterium salinarium (halobium). In some other strains, other sets of rhodopsin pumps and sensors have been found. Here, these bacterial rhodopsins are classified according to their amino acid sequence homologies, and their host genera are assigned on the basis of 16S rRNA sequence comparison. Haloarcula is the host for cruxrhodopsins and a new genus (temporarily "Halorubra") is the host for archaerhodopsins. Difference in the all-trans:13-cis ratios of retinal in two proton pumps (**bacteriorhodopsin** and archaerhodopsin-2) at equilibrium states in the dark was ascribed to only one amino acid residue in the retinal pocket. This predicted methionine-145 in **bacteriorhodopsin** was point-mutated to phenylalanine as in archaerhodopsin-2. The mutated **bacteriorhodopsin** (M145F) became to show the same dark-adapted isomer ratio that archaerhodopsin-2 shows. **Chimeric** proton pumps were made by exchanging genes of one or more helix regions of two similar pumps (archaerhodopsin-1 and -2) in order to know structural delicacy of the inter-helix space. Preliminary results show that some photochemical properties depend on one helix or one distinct amino acid residue on the helix. Such new lines initiated by our archaerhodopsins are discussed for studying structure and function of these unique bacterial rhodopsins.

L8 ANSWER 18 OF 61 MEDLINE
ACCESSION NUMBER: 94179130 MEDLINE
DOCUMENT NUMBER: 94179130 PubMed ID: 8132488
TITLE: Phosphate transport in mitochondria: past accomplishments, present problems, and future challenges.
AUTHOR: Ferreira G C; Pedersen P L
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, College of Medicine, University of South Florida, Tampa 33612.
SOURCE: JOURNAL OF BIOENERGETICS AND BIOMEMBRANES, **(1993 Oct)** 25 (5) 483-92. Ref: 58
Journal code: HIO; 7701859. ISSN: 0145-479X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404

ENTRY DATE: Entered STN: 19940428
Last updated on STN: 19940428
Entered Medline: 19940421

AB The requirement of inorganic phosphate (Pi) for oxidative phosphorylation in eukaryotic cells is fulfilled through specific Pi transport systems. The mitochondrial proton/phosphate symporter (Pic) is a membrane-embedded protein which translocates Pi from the cytosol into the mitochondrial matrix. Pic is responsible for the very rapid transport of most of the Pi used in ATP synthesis. During the past five years there have been advances on several fronts. Genomic and cDNA clones for yeast, bovine, rat, and human Pic have been isolated and sequenced. Functional expression of yeast Pic in yeast strains deficient in Pi transport and expression in Escherichia coli of a **chimera** protein involving Pic and ATP synthase alpha subunit have been accomplished. Pic, in contrast to other members of the family of transporters involved in energy metabolism, was demonstrated to have a presequence, which optimizes the import of the precursor protein into mitochondria. Six transmembrane segments appear to be a structural feature shared between Pic and other mitochondrial anion carriers, and recent-site directed mutagenesis studies implicate structure-functional relationships to **bacteriorhodopsin**. These recent advances on Pic will be assessed in light of a more global interpretation of transport mechanism across the inner mitochondrial membrane.

L8 ANSWER 19 OF 61 MEDLINE
ACCESSION NUMBER: 94134061 MEDLINE
DOCUMENT NUMBER: 94134061 PubMed ID: 8302281
TITLE: Intramolecular interactions in muscarinic acetylcholine receptors studied with **chimeric** m2/m5 receptors.
AUTHOR: Pittel Z; Wess J
CORPORATE SOURCE: National Institute of Diabetes and Digestive and Kidney Diseases, Laboratory of Bioorganic Chemistry, Bethesda, Maryland 20892.
SOURCE: MOLECULAR PHARMACOLOGY, **(1994 Jan)** 45 (1) 61-4.
Journal code: NGR; 0035623. ISSN: 0026-895X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199403
ENTRY DATE: Entered STN: 19940318
Last Updated on STN: 19980206
Entered Medline: 19940310

AB Current models of the three-dimensional structures of muscarinic acetylcholine receptors and other G protein-coupled receptors are based primarily on high-resolution electron diffraction data obtained with **bacteriorhodopsin**, the molecular structure of which is characterized by the presence of seven alpha-helical transmembrane domains (TM I-VII). However, **bacteriorhodopsin** does not couple to G proteins and its primary sequence lacks a series of amino acids that are conserved among virtually all G protein-coupled receptors. Therefore, it remains to be shown experimentally whether the molecular structures of these functionally different proteins are in fact identical. To address this question, we have analyzed the pharmacological properties of a series of hybrid human m2/m5 muscarinic receptors. Initially, we identified several **chimeric** constructs that, upon transient expression in COS-7 cells, were unable to bind significant amounts of the muscarinic antagonists N-[3H]methylscopolamine and [3H]quinuclidinyl benzilate. A common structural feature of these constructs was the presence of m2 receptor sequence in TM VII and of m5 receptor sequence in TM I. The ligand-binding activity of these "pharmacologically inactive" hybrid receptors could be restored by replacing TM I (consisting of m5 receptor sequence) with the corresponding m2 receptor domain. These data provide the first direct experimental evidence that the molecular architecture of muscarinic receptors (and, most likely, that of other G protein-coupled

receptors) resembl[redacted] that of **bacteriorhodopsin**, in [redacted]t the seven TM helices a[redacted] arranged in a ring-like fashion such that TM I lies directly adjacent to TM VII.

L8 ANSWER 20 OF 61 MEDLINE
ACCESSION NUMBER: 94112141 MEDLINE
DOCUMENT NUMBER: 94112141 PubMed ID: 8284326
TITLE: Analyzing the red-shift characteristics of azulenic, naphthyl, other ring-**fused** and retinyl pigment analogs of **bacteriorhodopsin**.
AUTHOR: Liu R S; Krogh E; Li X Y; Mead D; Colmenares L U; Thiel J R; Ellis J; Wong D; Asato A E
CORPORATE SOURCE: Department of Chemistry, University of Hawaii, Honolulu 96822.
CONTRACT NUMBER: DK-17806 (NIDDK)
SOURCE: PHOTOCHEMISTRY AND PHOTOBIOLOGY, (1993 Nov) 58 (5) 701-5.
Journal code: P69; 0376425. ISSN: 0031-8655.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940228
Last Updated on STN: 19940228
Entered Medline: 19940214

AB Prompted by the near infrared-absorbing properties of some of the azulenic **bacteriorhodopsin** (bR) analogs, we have analyzed their absorption characteristics along with 11 new related ring-**fused** analogs and the corresponding Schiff bases (SB) and protonated Schiff bases (PSB). The following three factors are believed to contribute to the total red shift of each of the pigment analogs (sigma RS): perturbation of the basic chromophore (SB shift, delta SB), protonation of the SB (PSB shift, PSBS) and protein perturbation (the opsin shift, OS). For each factor, effects of structural modifications were examined. For the red-shifted pigments, percent OS has been suggested as an alternate way of measuring protein perturbation. Computer-simulated chromophores provided evidence against any explanation involving altered shapes of the binding pocket as a major cause for absorption differences. Implications of the current bR results on preparation of further red-shifted bR and possible application to visual pigment analogs are discussed.

L8 ANSWER 21 OF 61 MEDLINE
ACCESSION NUMBER: 94076353 MEDLINE
DOCUMENT NUMBER: 94076353 PubMed ID: 8254676
TITLE: Projection structure of halorhodopsin from Halobacterium halobium at 6 A resolution obtained by electron cryo-microscopy.
AUTHOR: Havelka W A; Henderson R; Heymann J A; Oesterhelt D
CORPORATE SOURCE: Max-Planck-Institut fur Biochemie, Martinsried, Germany.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, **(1993 Dec 5)** 234 (3) 837-46.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940203
Last Updated on STN: 19940203
Entered Medline: 19940112

AB Two-dimensional crystals of halorhodopsin (HR), in space group p42(1)2 (a = 102 A) have been obtained using the overexpressing Halobacterium halobium strain D2. An HR membrane fraction with the same buoyant density as purple membrane (HR-PM) was obtained by homogenization and sucrose gradient purification and used for electron cryomicroscopic analysis. Electron micrographs and electron diffraction patterns of HR-PM were

recorded at liquid [redacted]trogen temperatures. The micro[redacted]hs showed significant diffraction out to 9 A resolution optically and to 6 A after computer processing. By combining data from electron micrographs and electron diffraction patterns, a projection map of HR was calculated. The crystal form of the isolated HR consists of one membrane in which alternating halorhodopsin tetramers are oriented in opposite directions across the membrane. It is not known whether this occurs by misinsertion of some of the molecules in vivo, or by adventitious **fusion** at some point during isolation. The projected structure of the HR molecule to a resolution of 6A is almost identical to that found for **bacteriorhodopsin** (BR). This physical structural similarity thus complements the known sequence relatedness to BR.

L8 ANSWER 22 OF 61 MEDLINE
ACCESSION NUMBER: 94074539 MEDLINE
DOCUMENT NUMBER: 94074539 PubMed ID: 7504623
TITLE: Properties of **bacteriorhodopsin** derivatives constructed by insertion of an exogenous epitope into extra-membrane loops.
COMMENT: Erratum in: EMBO J 1994 Dec 1;13(23):5794
AUTHOR: Teufel M; Pompejus M; Humbel B; Friedrich K; Fritz H J
CORPORATE SOURCE: Institut fur Molekulare Genetik, Georg-August-Universitat Gottingen, Germany.
SOURCE: EMBO JOURNAL, **(1993 Sep)** 12 (9) 3399-408.
Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940203
Last Updated on STN: 19970203
Entered Medline: 19940110

AB **Bacteriorhodopsin** (BR) is folded into a bundle of seven alpha-helices which is embedded in the cellular membrane of Halobacterium salinarium; these helices are connected by short extra-membrane loops, three on the cytoplasmic side and three on the outside. Oligonucleotide-directed insertion or replacement mutagenesis was used to integrate the C-terminal sequence (13 amino acids long) of Sendai virus L-protein individually into each of the six helix-connecting loops. The altered gene products were obtained by expression of the mutant genes in either Escherichia coli or Schizosaccharomyces pombe and were used to reconstitute BR in proteoliposomes. In four cases (altered loops B/C, C/D, D/E or E/F), the mutant BRs were found to be fully functional as judged by light-driven proton pumping and photocycle kinetics. Within the four functional BR variants, recognition of the viral epitope by a monoclonal antibody is restricted to modified loops B/C and E/F. Immunogold staining of S.pombe cells producing either of the two latter BR variants shows that the protein is distributed among various cellular membranes but is not present in mitochondrial membranes. Sequence alteration of loop A/B or F/G resulted in loss of function, most plausibly due to a folding defect of the respective proteins. These results on the one hand document differences in structural importance of the various BR extra-membrane loops and on the other hand open the door to the construction of multifunctional membrane proteins via loop replacement mutagenesis of BR.

L8 ANSWER 23 OF 61 MEDLINE
ACCESSION NUMBER: 93368462 MEDLINE
DOCUMENT NUMBER: 93368462 PubMed ID: 8395638
TITLE: Insertion of lipids and proteins into bacterial membranes by **fusion** with liposomes.
AUTHOR: Driessen A J; Konings W N
CORPORATE SOURCE: Department of Microbiology, University of Groningen, Haren,

The Netherlands.
SOURCE: METHODS IN ENZYMOLOGY, **(1993)** 221 394-408.
Journal code: MVA; 0212271. ISSN: 0076-6879.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199309
ENTRY DATE: Entered STN: 19931015
Last Updated on STN: 19931015
Entered Medline: 19930930

L8 ANSWER 24 OF 61 MEDLINE
ACCESSION NUMBER: 93276332 MEDLINE
DOCUMENT NUMBER: 93276332 PubMed ID: 8503039
TITLE: Dimerization of glycophorin A transmembrane helices: mutagenesis and modeling.
AUTHOR: Engelman D M; Adair B D; Brunger A; Flanagan J M; Hunt J F;
Lemmon M A; Treutlein H; Zhang J
CORPORATE SOURCE: Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06511.
SOURCE: SOCIETY OF GENERAL PHYSIOLOGISTS SERIES, **(1993)** 48 11-21. Ref: 31
Journal code: UU2; 0433431. ISSN: 0094-7733.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199306
ENTRY DATE: Entered STN: 19930716
Last Updated on STN: 19930716
Entered Medline: 19930629

L8 ANSWER 25 OF 61 MEDLINE
ACCESSION NUMBER: 93211304 MEDLINE
DOCUMENT NUMBER: 93211304 PubMed ID: 8384688
TITLE: Homologous overexpression of a light-driven anion pump in an archaebacterium.
AUTHOR: Heymann J A; Havelka W A; Oesterhelt D
CORPORATE SOURCE: Max-Planck-Institut fur Biochemie, Martinsried, Germany.
SOURCE: MOLECULAR MICROBIOLOGY, **(1993 Feb)** 7 (4) 623-30.
Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 19930514
Last Updated on STN: 19930514
Entered Medline: 19930427

AB The retinal protein halorhodopsin (HR), a light-driven chloride pump from Halobacterium halobium, was homologously overexpressed in this archaebacterium. Two DNA expression systems differing in their promoter region were investigated. The halopsin, hop, promoter coupled to the hop gene gave an increased level of HR synthesis. However, the extent of expression was driven by the copy number of the shuttle vector and did not reach the magnitude of the bacterio-opsin, bop, promoter system. Employing a gene **fusion** approach, the promoter for the bop gene was used to drive expression of the hop gene. A shuttle vector containing a bop-hop-cartridge was transformed into a HR-deficient strain and blueish-coloured transformants were obtained. The bop promoter expressed HR to an extent where a specific membrane fraction resembled the crystalline purple membrane of BR in terms of the lipid to protein ratio. HR could, therefore, be easily isolated in a natural membrane-bound state.

This allows for dir[redacted] use in biophysical studies wi[redacted]t the application of detergents. This [redacted]s the first successful overexpr[redacted]ssion of a 7-helical transmembrane protein and may be extended to other proteins of this family.

L8 ANSWER 26 OF 61 MEDLINE
ACCESSION NUMBER: 93154328 MEDLINE
DOCUMENT NUMBER: 93154328 PubMed ID: 8428583
TITLE: The SecA and SecY subunits of translocase are the nearest neighbors of a translocating preprotein, shielding it from phospholipids.
AUTHOR: Joly J C; Wickner W
CORPORATE SOURCE: Molecular Biology Institute, University of California, Los Angeles 90024-1570.
SOURCE: EMBO JOURNAL, **(1993 Jan)** 12 (1) 255-63.
Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930326
Last Updated on STN: 19930326
Entered Medline: 19930310

AB To study the environment of a preprotein as it crosses the plasma membrane of Escherichia coli, unique cysteinyl residues were introduced into proOmpA and the genes for these mutant preproteins were fused to the gene of dihydrofolate reductase (Dhfr). A photoactivable, radiolabeled and reducible cross-linker was then attached to the unique cysteinyl residue of each purified protein. Partially translocated polypeptides were generated and arrested in their membrane transit by the folded structure of the dihydrofolate reductase domain. After photolysis to label their nearest neighbors and reduction of the disulfide bond between proOmpA-Dhfr and the cross-linker, radiolabeled cross-linker was selectively recovered with the SecA and SecY subunits of preprotein translocase. Strikingly, neither the SecE nor Band 1 subunits were cross-linked to any of the constructs and the membrane phospholipids were almost entirely shielded from cross-linking. The fact that SecY and SecA are the only membrane proteins cross-linked to the translocating chains suggests that they may form an entirely proteinaceous pathway through which secreted proteins pass during membrane transit.

L8 ANSWER 27 OF 61 MEDLINE
ACCESSION NUMBER: 93145957 MEDLINE
DOCUMENT NUMBER: 93145957 PubMed ID: 7916683
TITLE: High-yield production of **bacteriorhodopsin** via expression of a synthetic gene in Escherichia coli.
AUTHOR: Pompejus M; Friedrich K; Teufel M; Fritz H J
CORPORATE SOURCE: Institute fur Molekulare Genetik, Georg-August-Universitat Gottingen, Federal Republic of Germany.
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, **(1993 Jan 15)** 211 (1-2) 27-35.
Journal code: EMZ; 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L09679; GENBANK-L09680; GENBANK-L09681; GENBANK-L09682; GENBANK-L09683; GENBANK-L16554; GENBANK-L16555; GENBANK-L16556; GENBANK-X70259; GENBANK-Z18286
ENTRY MONTH: 199302
ENTRY DATE: Entered STN: 19930312
Last Updated on STN: 19950206
Entered Medline: 19930226

AB A gene (bos) coding for bacterioopsin (BO), the apoprotein of

bacteriorhodopsin w[redacted]assembled from chemically synt[redacted]ized oligonucleotides by a new method of repeated rounds of insertion mutagenesis. The gene sequence was designed for convenient manipulation in future protein engineering experiments. In-frame **fusion** of bos to the lacZ454 gene allowed high-yield production in Escherichia coli of a beta-Gal454/BO **fusion** protein, deposited as intracellular inclusion bodies. These were enriched by virtue of their insolubility in 0.5% Triton X-100 and cleaved in aqueous suspension with IgA protease at a specific site provided at the beta-Gal454/BO boundary. Pure BO could be obtained from the mixture of water-insoluble cleavage products by selective extraction into organic solvent. The yield was in the range 30-50 mg pure protein/l culture medium, depending on individual preparation. This material could be used for reconstitution of fully functional **bacteriorhodopsin**. Taken together, the procedure constitutes a practical basis for the production of genetically engineered bacteriorhodopsins.

L8 ANSWER 28 OF 61 MEDLINE
ACCESSION NUMBER: 93054467 MEDLINE
DOCUMENT NUMBER: 93054467 PubMed ID: 1331041
TITLE: Identification of intramolecular interactions in adrenergic receptors.
AUTHOR: Suryanarayana S; von Zastrow M; Kobilka B K
CORPORATE SOURCE: Howard Hughes Medical Institute, Stanford University Medical Center, California 94305.
CONTRACT NUMBER: 5 RO1 NS28471 (NINDS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1992 Nov 5)** 267 (31) 21991-4.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19930122
Entered Medline: 19921201

AB Adrenergic receptors are representative of a large family of plasma membrane receptors that interact with G proteins during the process of transmembrane signal transduction. G protein-coupled receptors have a primary structure that is homologous to **bacteriorhodopsin** and are proposed to have a similar three-dimensional structure; however, it has not yet been possible to examine this hypothesis experimentally. We have used a novel mutagenesis approach to identify intramolecular interactions. Our results indicate that specific amino acids in the seventh hydrophobic segment of alpha 2 and beta 2 adrenergic receptors lie adjacent to the first hydrophobic segment. These studies provide the first experimental evidence defining spatial relationships that exist in the three-dimensional structure of adrenergic receptors.

L8 ANSWER 29 OF 61 MEDLINE
ACCESSION NUMBER: 92329435 MEDLINE
DOCUMENT NUMBER: 92329435 PubMed ID: 1627558
TITLE: **Bacteriorhodopsin** can be refolded from two independently stable transmembrane helices and the complementary five-helix fragment.
AUTHOR: Kahn T W; Engelman D M
CORPORATE SOURCE: Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06511.
CONTRACT NUMBER: GM22778 (NIGMS)
GM39546 (NIGMS)
SOURCE: BIOCHEMISTRY, **(1992 Jul 7)** 31 (26) 6144-51.

Journal code: A0G; 0370623. ISSN: 00[redacted]2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920904
Last Updated on STN: 19920904
Entered Medline: 19920818

AB This paper describes experimental tests of the hypothesis that **bacteriorhodopsin** (BR) can fold by the association of independently stable transmembrane helices. Peptides containing the first and second helical segments of BR were chemically synthesized. These two peptides and the complementary five-helix fragment of BR were reconstituted in three separate populations of native-lipid vesicles which were then mixed and fused to allow the fragments to interact. After addition of retinal, absorption spectroscopy of the reconstituted BR and X-ray diffraction of two-dimensional crystals of this material showed that the native structure of BR was regenerated. The first two helices of BR can therefore be considered as independent folding domains, and covalent connections in the loops connecting the helices to each other and to the rest of the molecule are not essential for the appropriate association of the helices.

L8 ANSWER 30 OF 61 MEDLINE
ACCESSION NUMBER: 92250740 MEDLINE
DOCUMENT NUMBER: 92250740 PubMed ID: 1374417
TITLE: Immunological evidence for eight spans in the membrane domain of 3-hydroxy-3-methylglutaryl coenzyme A reductase: implications for enzyme degradation in the endoplasmic reticulum.
AUTHOR: Roitelman J; Olender E H; Bar-Nun S; Dunn W A Jr; Simoni R D
CORPORATE SOURCE: Department of Biological Sciences, Stanford University, California 94305.
CONTRACT NUMBER: HL-26502 (NHLBI)
SOURCE: JOURNAL OF CELL BIOLOGY, **(1992 Jun)** 117 (5) 959-73.
Journal code: HMV; 0375356. ISSN: 0021-9525.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920619
Last Updated on STN: 19960129
Entered Medline: 19920611

AB We have raised two monospecific antibodies against synthetic peptides derived from the membrane domain of the ER glycoprotein 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in the cholesterol biosynthetic pathway. This domain, which was proposed to span the ER membrane seven times (Liscum, L., J. Finer-Moore, R. M. Stroud, K. L. Luskey, M. S. Brown, and J. L. Goldstein. 1985. J. Biol. Chem. 260:522-538), plays a critical role in the regulated degradation of the enzyme in the ER in response to sterols. The antibodies stain the ER of cells and immunoprecipitate HMG-CoA reductase and HMGal, a **chimeric** protein composed of the membrane domain of the reductase fused to Escherichia coli beta-galactosidase, the degradation of which is also accelerated by sterols. We show that the sequence Arg224 through Leu242 of HMG-CoA reductase (peptide G) faces the cytoplasm both in cultured cells and in rat liver, whereas the sequence Thr284 through Glu302 (peptide H) faces the lumen of the ER. This indicates that a sequence between peptide G and peptide H spans the membrane of the ER. Moreover, by epitope tagging with peptide H, we show that the loop segment

connecting membran ans 3 and 4 is sequestered in lumen of the ER. These results demo ate that the membrane domain o HMG-CoA reductase spans the ER eight times and are inconsistent with the seven membrane spans topological model. The approximate boundaries of the proposed additional transmembrane segment are between Lys248 and Asp276. Replacement of this 7th span in HMGal with the first transmembrane helix of **bacteriorhodopsin** abolishes the sterol-enhanced degradation of the protein, indicating its role in the regulated turnover of HMG-CoA reductase within the endoplasmic reticulum.

L8 ANSWER 31 OF 61 MEDLINE
ACCESSION NUMBER: 92156172 MEDLINE
DOCUMENT NUMBER: 92156172 PubMed ID: 1740463
TITLE: The role of the membrane domain in the regulated degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase.
AUTHOR: Chun K T; Simoni R D
CORPORATE SOURCE: Department of Biological Sciences, Stanford University, California 94305-5020.
CONTRACT NUMBER: GM07276 (NIGMS)
HL26502 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1992 Feb 25)** 267 (6) 4236-46.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199203
ENTRY DATE: Entered STN: 19920410
Last Updated on STN: 19920410
Entered Medline: 19920324

AB We have constructed a series of mutations in the membrane and linker domains of Syrian hamster 3-hydroxy-3-methylglutaryl-(HMG) CoA reductase in order to determine the regions critical for the regulated degradation of the enzyme. In transfected Chinese hamster ovary cells, we have expressed a **fusion** protein, HMGal, which consists of the membrane and linker domains of the Syrian hamster HMG-CoA reductase fused to beta-galactosidase. Using this **fusion** protein, we have determined that a deletion of 64 amino acids from the central region of the membrane domain causes the protein to be degraded extremely rapidly. In addition, deletion of PEST sequences has little effect on degradation, but deletion of the linker domain makes the protein's degradation insensitive to sterols and mevalonate. In addition to deletion mutations, we have systematically replaced each hydrophobic, putative membrane spanning region of the membrane domain with the first transmembrane sequence from **bacteriorhodopsin**. Replacement of span 4 has no effect on degradation. Replacements of spans 5 or 6 result in a protein which has a normal basal rate of degradation, but this rate of degradation is not accelerated by mevalonate, low density lipoprotein, or 25-hydroxycholesterol. Replacement of span 3 results in a protein whose degradation is similarly not accelerated by sterols or mevalonate, but since this protein might be mislocalized, these results are inconclusive. Replacement of span 7 yields a short-lived protein which is degraded more rapidly in response to mevalonate but not in response to exogenous sterols. Replacement of span 8 extends both the basal and mevalonate-accelerated half-life about 5-fold. This work begins to define the critical regions for regulated degradation within the membrane domain of HMG-CoA reductase.

L8 ANSWER 32 OF 61 MEDLINE
ACCESSION NUMBER: 91175762 MEDLINE
DOCUMENT NUMBER: 91175762 PubMed ID: 1848786
TITLE: Wild-type and mutant bacteriorhodopsins D85N, D96N, and R82Q: purification to homogeneity, pH dependence of pumping, and electron diffraction.
AUTHOR: Miercke L J; Betlach M C; Mitra A K; Shand R F; Fong S K; Stroud R M

CORPORATE SOURCE: Depa[redacted]ent of Biochemistry and Biophy , University of Cali[redacted]nia, San Francisco 94143-0448.
CONTRACT NUMBER: 5T32CA09043 (NCI)
GM31785 (NIGMS)
GM32079 (NIGMS)
SOURCE: BIOCHEMISTRY, **(1991 Mar 26)** 30 (12) 3088-98.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199105
ENTRY DATE: Entered STN: 19910519
Last Updated on STN: 19910519
Entered Medline: 19910501

AB Bacterioopsin, expressed in Escherichia coli as a **fusion** protein with 13 heterologous residues at the amino terminus, has been purified in the presence of detergents and retinylated to give **bacteriorhodopsin**. Further purification yielded pure **bacteriorhodopsin**, which had an absorbance ratio (A280/A lambda max) of 1.5 in the dark-adapted state in a single-detergent environment. This protein has a folding rate, absorbance spectrum, and light-induced proton pumping activity identical with those of **bacteriorhodopsin** purified from Halobacterium halobium. Protein expressed from the mutants D85N, D96N, and R82Q and purified similarly yielded pure protein with absorbance ratios of 1.5. Proton pumping rates of bacteriorhodopsins with the wild-type sequence and variants D85N, D96N, and R82Q were determined in phospholipid vesicles as a function of pH. D85N was inactive at all pH values, whereas D96N was inactive from pH 7.0 to pH 8.0, where wild type is most active, but had some activity at low pH. R82Q showed diminished proton pumping with the same pH dependence as for wild type. **Bacteriorhodopsin** purified from E. coli crystallized in two types of two-dimensional crystal lattices suitable for low-dose electron diffraction, which permit detailed analysis of structural differences in site-directed variants. One lattice was trigonal, as in purple membrane, and showed a high-resolution electron diffraction pattern from glucose-sustained patches. The other lattice was previously uncharacterized with unit cell dimensions a = 127 A, b = 67 A, and symmetry of the orthorhombic plane group pgg.

L8 ANSWER 33 OF 61 MEDLINE
ACCESSION NUMBER: 91175761 MEDLINE
DOCUMENT NUMBER: 91175761 PubMed ID: 2007142
TITLE: Wild-type and mutant bacterioopsins D85N, D96N, and R82Q: high-level expression in Escherichia coli.
AUTHOR: Shand R F; Miercke L J; Mitra A K; Fong S K; Stroud R M; Betlach M C
CORPORATE SOURCE: Department of Biochemistry and Biophysics, University of California, San Francisco 94143-0448.
CONTRACT NUMBER: 5T32CA09043 (NCI)
GM31785 (NIGMS)
GM32079 (NIGMS)
SOURCE: BIOCHEMISTRY, **(1991 Mar 26)** 30 (12) 3082-8.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199105
ENTRY DATE: Entered STN: 19910519
Last Updated on STN: 19910519
Entered Medline: 19910501

AB The integral membrane protein bacterioopsin, found in the extremely halophilic archaebacterium Halobacterium halobium, was expressed in Escherichia coli as a **fusion** protein containing 13 heterologous amino acids at the amino terminus. The expressed protein was localized primarily to the E. coli cytoplasmic membrane (greater than 80%) and had an in vivo half-life of 26 min. The amount of bacterioopsin in E. coli crude lysates was quantitated immunologically from Western blots and was

expressed at 10-20-fold higher levels than seen previously (i.e., 17 mg/L;
5.6% of the total protein). Three distinct forms of the protein were detected immunologically: two of the forms were generated by the removal of either one or four amino acid residues at the amino terminus; the third
form remained unaltered.

L8 ANSWER 34 OF 61 MEDLINE
ACCESSION NUMBER: 89283728 MEDLINE
DOCUMENT NUMBER: 89283728 PubMed ID: 2472007
TITLE: Purification and reconstitution of chloride channels from kidney and trachea.
AUTHOR: Landry D W; Akabas M H; Redhead C; Edelman A; Cragoe E J Jr; Al-Awqati Q
CORPORATE SOURCE: Department of Medicine, College of Physicians and Surgeons,
Columbia University, New York, NY 10032.
CONTRACT NUMBER: DK-20999 (NIDDK)
DK-39532 (NIDDK)
DK-41146 (NIDDK)
+
SOURCE: SCIENCE, **(1989 Jun 23)** 244 (4911) 1469-72.
Journal code: UJ7; 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198907
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19970203
Entered Medline: 19890724

AB Chloride channels mediate absorption and secretion of fluid in epithelia, and the regulation of these channels is now known to be defective in cystic fibrosis. Indanyl-oxyacetic acid 94 (IAA-94) is a high-affinity ligand for the chloride channel, and an affinity resin based on that structure was developed. Solubilized proteins from kidney and trachea membranes were applied to the affinity matrix, and four proteins with apparent molecular masses of 97, 64, 40, and 27 kilodaltons were eluted from the column by excess IAA-94. A potential-dependent 36Cl- uptake was observed after reconstituting these proteins into liposomes. Three types of chloride channels with single-channel conductances of 26, 100, and 400 picosiemens were observed after **fusion** of these liposomes with planar lipid bilayers. Similar types of chloride channels have been observed in epithelia.

L8 ANSWER 35 OF 61 MEDLINE
ACCESSION NUMBER: 89054035 MEDLINE
DOCUMENT NUMBER: 89054035 PubMed ID: 3142879
TITLE: Reconstitution of membrane proteins. Spontaneous incorporation of integral membrane proteins into preformed bilayers of pure phospholipid.
AUTHOR: Scotto A W; Zakim D
CORPORATE SOURCE: Department of Medicine, Cornell University Medical College,
New York, New York 10021.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1988 Dec 5)** 263 (34) 18500-6.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198901
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19890103

AB The spontaneous reconstitution of lipid-protein complexes was examined by mixing **bacteriorhodopsin** or UDP-glucuronosyltransferase with

preformed, unilamellar bilayers of pure dimyristoylphosphatidylcholine. Spontaneous insertion of these proteins into vesicles of dimyristoylphosphatidylcholine was facilitated by resonicating the vesicles at 4 degrees C. The property of resonicated vesicles that led to spontaneous reconstitution could be annealed by melting the bilayers, which slowed down reconstitution. The overall process of reconstitution consisted, however, of two steps. There was an initial insertion of proteins into a small portion of vesicles followed by subsequent **fusion** between protein-free vesicles and vesicles containing lipid-protein complexes. The first step appeared to proceed rapidly in all vesicles in a gel phase, whether or not they were resonicated or whether or not resonicated vesicles were annealed. The rate of the second step was sensitive to these treatments. The membrane proteins also inserted into preformed vesicles in a liquid crystalline phase, but this step was slower than for vesicles in a gel phase. **Fusion** between protein-free and protein-containing vesicles in a liquid crystalline phase was extremely slow. The data show that the spontaneous insertion of pure membrane proteins into preformed vesicles can be a facile event and that the overall reconstitution of membrane proteins into preformed unilamellar vesicles may be simpler to achieve than has been appreciated.

L8 ANSWER 36 OF 61 MEDLINE

ACCESSION NUMBER: 89017235 MEDLINE
DOCUMENT NUMBER: 89017235 PubMed ID: 2845411
TITLE: Human beta 2-adrenergic receptors expressed in Escherichia coli membranes retain their pharmacological properties.
AUTHOR: Marullo S; Delavier-Klutchko C; Eshdat Y; Strosberg A D; Emorine L
CORPORATE SOURCE: Centre National de la Recherche Scientifique, Universite Paris VII, France.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1988 Oct)** 85 (20) 7551-5.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198811
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19881121

AB The coding region of the gene for the human beta 2-adrenergic receptor gene was fused to the beta-galactosidase gene of the lambda gt11 expression vector. The Y1089 Escherichia coli strain was lysogenized with this modified vector and transcription of the **fusion** gene was induced. Expression of this transcription unit was shown by the appearance in the bacteria of proteins of molecular weight higher than that of native beta-galactosidase, which are immunoreactive with anti-beta-galactosidase antibodies. Production of beta 2-adrenergic receptors was shown by the presence, on intact bacteria, of binding sites for catecholamine agonists and antagonists possessing a typical beta 2-adrenergic pharmacological profile. Binding and photoaffinity labeling studies performed on intact E. coli and its membrane fractions showed that these binding sites are located in the inner membrane of the bacteria. Expression of pharmacologically active human beta 2-adrenergic receptors in E. coli further supports the similar transmembrane organization proposed for **bacteriorhodopsin** and eukaryotic membrane-embedded receptors coupled to guanine nucleotide-binding regulatory proteins. Moreover, this system should facilitate future analyses of the ligand-binding properties within this family of membrane receptors.

L8 ANSWER 37 OF 61 MEDLINE
ACCESSION NUMBER: 88118961 MEDLINE
DOCUMENT NUMBER: 88118961 PubMed ID: 3430624
TITLE: Refolding of **bacteriorhodopsin** in lipid bilayers. A thermodynamically controlled two-stage process.
AUTHOR: Popot J L; Gerchman S E; Engelman D M
CORPORATE SOURCE: Department of Molecular Biochemistry and Biophysics, Yale University, New Haven, CT 06511.
CONTRACT NUMBER: AI20466 (NIAID)
GM 22778 (NIGMS)
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, **(1987 Dec 20)** 198 (4) 655-76.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198803
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19880324

AB Possible steps in the folding of **bacteriorhodopsin** are revealed by studying the refolding and interaction of two fragments of the molecule reconstituted in lipid vesicles. (1) Two denatured **bacteriorhodopsin** fragments have been purified starting from chymotryptically cleaved **bacteriorhodopsin**. Cleaved **bacteriorhodopsin** has been renatured from a mixture of the fragments in Halobacterium lipids/retinal/dodecyl sulfate solution following removal of dodecyl sulfate by precipitation with potassium. The renatured molecules have the same absorption spectrum and extinction coefficient as native cleaved **bacteriorhodopsin**. They are integrated into small lipid vesicles as a mixture of monomers and aggregates. Extended lattices form during the partial dehydration process used to orient samples for X-ray and neutron crystallography. (2) Correct refolding of cleaved bacterioopsin occurs upon renaturation in the absence of retinal. Regeneration of the chromophore and reformation of the purple membrane lattice are observed following subsequent addition of all-trans retinal. (3) The two chymotryptic fragments have been reinserted separately into lipid vesicles and refolded in the absence of retinal. Circular dichroism spectra of the polypeptide backbone transitions indicate that they have regained a highly alpha-helical structure. The kinetics of chromophore regeneration following reassociation have been studied by absorption spectroscopy. Upon vesicle **fusion**, the refolded fragments first reassociate, then bind retinal and finally regenerate cleaved **bacteriorhodopsin**. The complex formed in the absence of retinal is kinetically indistinguishable from cleaved bacterioopsin. The refolded fragments in lipid vesicles are stable for months, both as separate entities and after reassociation. These observations provide further evidence that the native folded structure of **bacteriorhodopsin** lies at a free energy minimum. They are interpreted in terms of a two-stage folding mechanism for membrane proteins in which stable transmembrane helices are first formed. They subsequently pack without major rearrangement to produce the tertiary structure.

L8 ANSWER 38 OF 61 MEDLINE
ACCESSION NUMBER: 88032927 MEDLINE
DOCUMENT NUMBER: 88032927 PubMed ID: 2822680
TITLE: A mechanism of respiratory control: studies with proteoliposomes containing cytochrome oxidase and **bacteriorhodopsin**.
AUTHOR: Miki T; Orii Y; Mukohata Y
CORPORATE SOURCE: Department of Public Health, Faculty of Medicine, Kyoto University.
SOURCE: JOURNAL OF BIOCHEMISTRY, (1987 Jul) 102 (1) 199-209.
Journal code: HIF; 0376600. ISSN: 0021-924X.
PUB. COUNTRY: Japan

Jou[redacted]l; Article; (JOURNAL ARTICLE)
LANGUAGE: Eng[redacted]h
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198712
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19871214

AB Both beef heart cytochrome oxidase and **bacteriorhodopsin** of Halobacterium halobium were reconstituted into liposomes by the sonication-cholate dialysis method. The proteoliposomes showed the respiratory control ratio of 4.2, and steady-state illumination of the vesicles lead to the 2.7-fold stimulation of the oxidase activity in the absence of uncouplers. The light-stimulated state 4 respiration increased with light intensity, but light had no effect on the oxidase activity that had been relieved by addition of uncouplers. Proteoliposomes with the photosensitive oxidase activity were also obtained when cytochrome oxidase vesicles were **fused** with **bacteriorhodopsin** vesicles in the presence of calcium chloride, and the extent of photoactivation was maximally 1.4-fold. The light-induced respiratory release was observed even in the presence of valinomycin or nigericin, indicating that the oxidase activity was sensitive to both the membrane potential and the pH gradient. We propose as a mechanism of the respiratory control that the process of proton transport to the reaction center for water formation is the rate limiting step for the cytochrome oxidase activity.

L8 ANSWER 39 OF 61 MEDLINE
ACCESSION NUMBER: 87250573 MEDLINE
DOCUMENT NUMBER: 87250573 PubMed ID: 3298252
TITLE: Structure-function studies on **bacteriorhodopsin**. I. Expression of the bacterio-opsin gene in Escherichia coli.
AUTHOR: Dunn R J; Hackett N R; McCoy J M; Chao B H; Kimura K; Khorana H G
CONTRACT NUMBER: AI-11479 (NIAID)
GM28289-06 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1987 Jul 5)** 262 (19) 9246-54.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198708
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19870814

AB To express the bacterio-opsin (bop) gene in Escherichia coli, we have employed the inducible expression vectors pIN-II-A, -B, and -C (Nakamura, K., and Inouye, M. (1982) EMBO J. 1, 771-775). The vectors contain three cloning sites early in the E. coli lipoprotein gene (lpp) which is transcribed from tandem lpp and lac promoters. The bop gene was modified so as to delete the N-terminal leader sequence and then cloned into each of the three cloning sites to encode three different lipoprotein/bacterio-opsin fusions. Expression of the fusions was demonstrated both in vitro and in vivo. The **fusion** protein was estimated to be about 0.05% of the total cell protein. The cause for the low level of expression apparently was neither an inadequate level of mRNA nor degradation of the protein. However, expression of the fusions caused inhibition of the growth of the host to varying extents. One **fusion** protein was purified from E. coli membranes to homogeneity by immunoaffinity chromatography followed by preparative gel electrophoresis. The purified **fusion** protein generated a **bacteriorhodopsin**-like chromophore on treatment with defined lipid/detergent mixtures and retinal. When reconstituted into vesicles, the protein pumped protons on illumination comparably to the reconstituted native bacterio-opsin.

L8 ANSWER 40 OF 61 MEDLINE
ACCESSION NUMBER: 86300711 MEDLINE
DOCUMENT NUMBER: 86300711 PubMed ID: 3017712
TITLE: Calcium transport in membrane vesicles of Streptococcus cremoris.
AUTHOR: Driessen A J; Konings W N
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, **(1986 Aug 15)** 159 (1) 149-55.
Journal code: EMZ; 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198610
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19861023

AB Rightside-out membrane vesicles of Streptococcus cremoris were fused with proteoliposomes containing the light-driven proton pump **bacteriorhodopsin** by a low-pH **fusion** procedure reported earlier [Driessen, A.J.M., Hellingwerf, K.J. & Konings, W.N. (1985) Biochim. Biophys. Acta 808, 1-12]. In these fused membranes a proton motive force, interior positive and acid, can be generated in the light and this proton motive force can drive the uptake of Ca2+. Collapsing delta psi with a concomitant increase in delta pH stimulates Ca2+ uptake while dissipation of the delta pH results in a reduced rate of Ca2+ uptake. Also an artificially generated delta pH, interior acid, can drive Ca2+ uptake in S. cremoris membrane vesicles. Ca2+ uptake depends strongly on the presence of external phosphate while Ca2+-efflux-induced proton flux is independent of the presence of external phosphate. Ca2+ accumulation is abolished by the divalent cation ionophore A23187. Calcium extrusion from intact cells is accelerated by lactose. Collapse of the proton motive force by the uncoupler carbonylcyanide p-trifluoromethoxyphenylhydrazone or inhibition of the membrane-bound ATPase by N,N'-dicyclohexylcarbodiimide strongly inhibits Ca2+ release. Further studies on Ca2+ efflux at different external pH values in the presence of either valinomycin or nigericin suggested that Ca2+ exit from intact cells is an electrogenic process. It is concluded that Ca2+ efflux in S. cremoris is mediated by a secondary transport system catalyzing exchange of calcium ions and protons.

L8 ANSWER 41 OF 61 MEDLINE
ACCESSION NUMBER: 86216055 MEDLINE
DOCUMENT NUMBER: 86216055 PubMed ID: 3011065
TITLE: Reconstitution of membrane proteins: catalysis by cholesterol of insertion of integral membrane proteins into preformed lipid bilayers.
AUTHOR: Scotto A W; Zakim D
CONTRACT NUMBER: S07 RR05396 (NCRR)
SOURCE: BIOCHEMISTRY, **(1986 Apr 8)** 25 (7) 1555-61.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198607
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19860701

AB The presence of cholesterol in small unilamellar vesicles (ULV) of dimyristoylphosphatidylcholine (DMPC) catalyzes **fusion** of the vesicles at temperatures below the upper limit for the gel to liquid-crystalline phase transition of the DMPC. The extent to which ULV grow depends on the concentration of cholesterol in the vesicles and on

temperature. Maximum growth occurs at 21 degrees C. [redacted] decreases as the temperature is lowered below 21 degrees C. Growth does not occur at temperatures above the phase transition. In addition, the presence of cholesterol in ULV of DMPC catalyzes the insertion of integral membrane proteins into the vesicles. Thus, **bacteriorhodopsin** from Halobacterium halobrium, UDPglucuronosyltransferase (EC 2.4.1.17) from pig liver microsomes, and cytochrome oxidase from beef heart mitochondria formed stable lipid-protein complexes spontaneously when added to ULV containing cholesterol at temperatures under which these vesicles would fuse. Incorporation of these proteins into the ULV of DMPC did not occur in the absence of cholesterol or in the presence of cholesterol when the temperature of the system was above that for the phase transition. It appears that cholesterol lowers the energy barrier for **fusion** of ULV of DMPC and for insertion of integral membrane proteins into these bilayers. Studies with **bacteriorhodopsin** suggest that the energy barrier for insertion of proteins into ULV containing cholesterol is smaller than the energy barrier for **fusion** of the ULV with each other.

L8 ANSWER 42 OF 61 MEDLINE
ACCESSION NUMBER: 86026247 MEDLINE
DOCUMENT NUMBER: 86026247 PubMed ID: 2996590
TITLE: Reconstitution of membrane proteins. Spontaneous association of integral membrane proteins with preformed unilamellar lipid bilayers.
AUTHOR: Scotto A W; Zakim D
SOURCE: BIOCHEMISTRY, **(1985 Jul 16)** 24 (15) 4066-75.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198512
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19851205

AB We have developed a simple method for reconstituting pure, integral membrane proteins into phospholipid-protein vesicles. The method does not depend on use of detergents or sonication. It has been used successfully with three different types of integral membrane proteins: UDPglucuronosyltransferase (EC 2.4.1.17) from pig liver microsomes, cytochrome oxidase (EC 1.9.3.1) from pig heart, and **bacteriorhodopsin** from Halobacterium halobium. The method depends on preparing unilamellar vesicles of dimyristoylphosphatidylcholine (DMPC) that contain a small amount of myristate as fusogen. Under conditions that the vesicles of DMPC have the property of fusing, all of the above proteins incorporated into bilayers. Two events appear to be involved in forming the phospholipid-protein complexes. The first is a rapid insertion of all proteins into a small percentage of total vesicles. The second is slower but continued **fusion** of the remaining phospholipid-protein vesicles, or proteoliposomes, with small unilamellar vesicles of DMPC. This latter process was inhibited by conditions under which vesicles of DMPC themselves would not fuse. On the basis of proton pumping by **bacteriorhodopsin** and negative staining, the vesicles were unilamellar and large. The data suggest that insertion of the above integral membrane proteins into vesicles occurred independently of **fusion** between vesicles.

L8 ANSWER 43 OF 61 MEDLINE
ACCESSION NUMBER: 83238465 MEDLINE
DOCUMENT NUMBER: 83238465 PubMed ID: 6305986
TITLE: Introduction and characterization of amber mutations in the **bacteriorhodopsin** gene.
AUTHOR: McCoy J M; Khorana H G

CONTRACT NUMBER: AI1[redacted]9 (NIAID)
GM2[redacted]9 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1983 Jul 10)** 258 (13) 8456-61.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198308
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19830817

AB A **fusion** between the genes for **bacteriorhodopsin** and beta-galactosidase was constructed on a multicopy plasmid, pXB/Gal 101. The **fusion** gene, containing the **bacteriorhodopsin** gene fused upstream from the beta-galactosidase gene, was under the control of tandem lipoprotein and lac gene promoters. When expressed in Escherichia coli the **fusion** protein retained beta-galactosidase activity. Mutations in the **fusion** gene were produced by passage of pXB/Gal 101 through the E. coli mutator strain mut D5. Amber mutations were then selected by examining the loss of the lac+ phenotype imparted by the **fusion** protein to lac- E. coli cells. Amber mutations occurring within the **bacteriorhodopsin** gene were localized by replacing the beta-galactosidase region of each mutant plasmid with a beta-galactosidase region which was known to be unmutated. Precise localization of the mutations was achieved first by sizing the prematurely terminated peptides produced by the mutant plasmids in in vitro coupled transcription-translation reactions, and secondly by DNA sequence analysis. Six amber mutants in the gene for **bacteriorhodopsin** were characterized in this way. One of these was a transversion mutation at a lysine codon; the other five were all transition mutations at tryptophan codons, codons 10, 12, 80, 86, and 137 of the **bacteriorhodopsin** sequence.

L8 ANSWER 44 OF 61 MEDLINE
ACCESSION NUMBER: 82150898 MEDLINE
DOCUMENT NUMBER: 82150898 PubMed ID: 6278476
TITLE: Transmembranous incorporation of photoelectrically active **bacteriorhodopsin** in planar lipid bilayers.
AUTHOR: Bamberg E; Dencher N A; Fahr A; Heyn M P
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1981 Dec) 78 (12) 7502-6.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198205
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19900317
Entered Medline: 19820521

AB Various methods to incorporate **bacteriorhodopsin** in black lipid membranes are reported. Both purple membrane patches and monomeric **bacteriorhodopsin** were used as starting material. The incorporation of **bacteriorhodopsin** into planar lipid bilayers was achieved by the following methods. (i) Purple membrane patches were transferred from water to solutions of lipids in n-alkanes. Black membranes were formed from such organic suspensions. (ii) Lipid layers containing solvent and purple membranes were spread on an air/water interface. These layers were used to form planar bilayers. (iii) Vesicles containing purple membranes or monomeric **bacteriorhodopsin** were spread on an air/water interface and, from the resulting layer, bilayers were formed. On illumination, steady-state photocurrents were observed in all three cases, indicating that these methods lead to functional transmembranous integration of the protein in the planar black lipid membrane. The influence of an applied electric field on the pumping process was studied on membranes formed by using method i. At

approximately 200 m[redacted] the photocurrent tends to zero. Furthermore, it was possible to make planar lipid bilayers photoelectrically active by adding vesicles containing monomeric **bacteriorhodopsin** to the bathing solution. Because, in this case, only transient photocurrents were observed, it can be concluded that the vesicles are attached to but not **fused** with the black lipid membrane.

L8 ANSWER 45 OF 61 MEDLINE
ACCESSION NUMBER: 82142410 MEDLINE
DOCUMENT NUMBER: 82142410 PubMed ID: 6174514
TITLE: Immunological probes for **bacteriorhodopsin**. Identification of three distinct antigenic sites on the cytoplasmic surface.
AUTHOR: Kimura K; Mason T L; Khorana H G
CONTRACT NUMBER: AI 11479 (NIAID)
GM 28289 (NIGMS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, **(1982 Mar 25)** 257 (6) 2859-67.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198205
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19970203
Entered Medline: 19820527

AB We have prepared site-specific immunological reagents to study the orientation and surface topography of the integral membrane protein **bacteriorhodopsin**. Monoclonal and polyclonal antibodies with strong affinity for antigenic determinants on proteolytic and cyanogen bromide fragments of **bacteriorhodopsin** have been isolated and characterized. Three distinct antibody binding sites have been identified on the cytoplasmic surface of **bacteriorhodopsin**. The first due is readily accessible in native **bacteriorhodopsin** and lies close to the COOH terminus. This binding site is lost when only three amino acid residues are removed from the COOH terminus. The second site, which is also near the COOH terminus, is located approximately within the 17 COOH terminal amino acid residues. The third site is in the fragment that comprises Tyr-83 to Met-118 and is probably contained in the short loop connecting the third and fourth helices. The use of COOH terminus-specific antibodies in determination of the orientation of **bacteriorhodopsin** molecules in the Halobacterium halobium membrane confirms the earlier conclusion that the COOH terminus is on the cytoplasmic side.

L8 ANSWER 46 OF 61 MEDLINE
ACCESSION NUMBER: 79124764 MEDLINE
DOCUMENT NUMBER: 79124764 PubMed ID: 84685
TITLE: Association of **bacteriorhodopsin** with lipid-impregnated filters. Evidence for **fusion** of **bacteriorhodopsin**-containing vesicles with the lipid phase of the filter.
AUTHOR: Blok M C; van Dam K
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, **(1979 Feb 2)** 550 (3) 527-42.
Journal code: A0W; 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197905
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19950206
Entered Medline: 19790516

AB **Bacteriorhodopsin** vesicles were associated with cellulose-nitrate filters impregnated with a solution of phospholipids in

hexadecane. The generation of (photo)potentials upon illumination of the filter was studied in the absence and presence of ionophores, phospholipase A2, EDTA or polyene antibiotics. From these experiments the following conclusions are drawn. 1. Upon illumination of the filter, **bacteriorhodopsin** pumps protons into aqueous compartments located in the filter. 2. These aqueous compartments possibly do not originate from the compartments enclosed by the **bacteriorhodopsin** vesicles. Evidence is obtained that aqueous compartments are present in the surface layers of the lipid-impregnated filters. 3. The results are explained most easily by a mechanism, whereby **fusion** occurs between the vesicles and the lipids of the filter.

L8 ANSWER 47 OF 61 MEDLINE
ACCESSION NUMBER: 78104315 MEDLINE
DOCUMENT NUMBER: 78104315 PubMed ID: 623861
TITLE: The electrical response to light of **bacteriorhodopsin** in planar membranes.
AUTHOR: Herrmann T R; Rayfield G W
SOURCE: BIOPHYSICAL JOURNAL, **(1978 Feb)** 21 (2) 111-25.
Journal code: A5S; 0370626. ISSN: 0006-3495.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197804
ENTRY DATE: Entered STN: 19900314
Last Updated on STN: 19970203
Entered Medline: 19780417

AB We have measured the light-induced short-circuit current generated by a planar membrane containing **bacteriorhodopsin** incorporated by vesicle **fusion**. The experimental results are consistent with an equivalent electrical circuit analogue that assumes that the vesicles remain intact after **fusion** and that the current generator equivalent of the light-driven proton pump is linearly dependent on bias voltage. The transient response to light of the planar membrane has also been examined. Slow response times are seen to be associated with the capacitive charging and discharging of the fused vesicles. A study of the leading edge of the light response curve of the planar membrane yields information about the transient response of the light-driven proton pump. We propose that the translocation of protons across the membrane is associated with a first-order process characterized by a rate constant lambda.

L8 ANSWER 48 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:688 BIOSIS
DOCUMENT NUMBER: PREV199800000688
TITLE: Proton translocation across **bacteriorhodopsin** containing solid supported lipid bilayers.
AUTHOR(S): Steinem, Claudia; Janshoff, Andreas; Hoehn, Fredrick; Sieber, Manfred; Galla, Hans-Joachim (1)
CORPORATE SOURCE: (1) Inst. Biochem., Westfaelische Wilhelms-Univ., Wilhelm-Klemm-Str. 2, D-48149 Muenster Germany
SOURCE: Chemistry and Physics of Lipids, (**Oct. 22, 1997**) Vol. 89, No. 2, pp. 141-152.
ISSN: 0009-3084.
DOCUMENT TYPE: Article
LANGUAGE: English

AB **Bacteriorhodopsin** (BR) was incorporated in solid supported lipid bilayers by **fusion** of reverse phase vesicles on chemisorbed monolayers of 1,2-dimyristoyl-sn-glycero-3-phosphothioethanol (DMPTE) on gold substrates. The passive electrical behavior of the artificial membranes was monitored by impedance spectroscopy in order to determine both the membrane resistances and capacitances and to guarantee reproducibility of the bilayer formation. Illumination of the BR containing solid supported lipid bilayers resulted in a transient photocurrent as expected from earlier experiments with black lipid membranes. The present preparation technique however is advantageous because of its long term stability up to 1 day without loss of BR activity

and its easy handl[redacted]. We investigated the dependen[redacted]of the photocurrent on the BR content, [redacted]pid environment, pH, and a proton carrier using a common current amplifier. Maximum current densities were obtained in the presence of negatively charged lipids like 1,2-dimyristoyl-sn-glycero-3-phosphatidic acid (DMPA) or 1-palmitoyl-2-sn-glycero-3-phosphoglycerol (POPG) at a pH of 6.4. Moreover it could be shown that the pump activity of reconstituted BR is insignificantly influenced by the capacitance of the first self-assembled DMPTE-monolayer on the gold electrodes. This may be explained by an incomplete **fusion** of BR containing vesicles on the hydrophobic surface. Carbonylcyanid-4-trifluoromethoxy-phenylhydrazone (FCCP), a membrane soluble proton translocator, increases the membrane conductance as well as the capacitance of the lipid bilayer that was derived either from impedance spectroscopy or evaluation of the time constants of the transient photocurrents.

L8 ANSWER 49 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:398706 BIOSIS
DOCUMENT NUMBER: PREV199598413006
TITLE: Properties of the peribacteroid membrane ATPase of pea root nodules and its effect on the nitrogenase activity.
AUTHOR(S): Szafran, Magdalena M.; Haaker, Huub (1)
CORPORATE SOURCE: (1) Dep. Biochem., Agric. Univ., Dreijenlaan 3, 6703 HA Wageningen Netherlands
SOURCE: Plant Physiology (Rockville), (1995) Vol. 108, No. 3, pp. 1227-1232.
ISSN: 0032-0889.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Peribacteroid membrane vesicles from pea (Pisum sativum) root nodules were isolated from membrane-enclosed bacteroids by an osmotic shock. The ATPase activity associated with this membrane preparation was characterized, and its electrogenic properties were determined. The pH gradient was measured as a change of the fluorescence intensity of 9-amino-6-chloro-2-methoxyacridine and the membrane potential as a shift of absorbance of bis-(3-propyl-5-oxoisoxazol-4-yl)pentamethine oxonol. it was demonstrated that the ATPase generates a pH gradient as well as a membrane potential across the peribacteroid membrane. The reversibility of the ATPase was demonstrated by a light-dependent ATP synthesis by peribacteroid membrane vesicles **fused** with **bacteriorhodopsin**-phospholipid vesicles. The light-driven ATP synthesis by the peribacteroid membrane ATPase was completely inhibited by a proton-conducting ionophore. The proton-pumping activity of the peribacteroid membrane ATPase could also be demonstrated with peribacteroid membrane-enclosed bacteroids, and effects on nitrogenase activity were established. At pH values below 7.5, an active peribacteroid membrane ATPase inhibited the nitrogenase activity of peribacteroid membrane-enclosed bacteroids. At pH values above 8, at which whole cell nitrogenase activity was inhibited, the proton-pumping activity of the peribacteroid membrane ATPase could partially reverse the pH inhibition. Vanadate, an inhibitor of plasma membrane and peribacteroid membrane ATPases, stimulated nodular nitrogenase activity. It will be proposed that the proton-pumping activity of the peribacteroid membrane ATPase in situ is a possible regulator of nodular nitrogenase activity.

L8 ANSWER 50 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:139328 BIOSIS
DOCUMENT NUMBER: PREV199598153628
TITLE: BR-ATCase: A **fusion** strategy for crystallization of membrane proteins.
AUTHOR(S): Turner, G. J.; Miercke, L. J.; Mitra, A.; Schafmeister, C.; Betlach, M.; Stroud, R. M.

CORPORATE SOURCE: Dep[redacted]iochem. Biophysics, UCSF, San [redacted]ncisco, CA 94143 USA
SOURCE: Biophysical Journal, (1995) Vol. 68, No. 2 PART 2, pp. A330.
Meeting Info.: 39th Annual Meeting of the Biophysical Society San Francisco, California, USA February 12-16, 1995
ISSN: 0006-3495.
DOCUMENT TYPE: Conference
LANGUAGE: English

L8 ANSWER 51 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1993:499151 BIOSIS
DOCUMENT NUMBER: PREV199396123158
TITLE: A C-terminal truncation results in high-level expression of the functional photoreceptor sensory rhodopsin I in the archaeon Halobacterium salinarium.
AUTHOR(S): Ferrando-May, Elisa (1); Brustmann, Bettina; Oesterhelt, Dieter
CORPORATE SOURCE: (1) Max-Planck-Inst. fuer Biochemie, D-82143 Martinsried Germany
SOURCE: Molecular Microbiology, (1993) Vol. 9, No. 5, pp. 943-953.
ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Expression of the gene encoding the halobacterial photoreceptor sensory rhodopsin I (SRI), sopl, was studied by means of homologous gene targeting. A sopl- Halobacterium salinarium mutant strain was constructed by homologous replacement of sopl with a novobiocin-resistant gyrB from Haloferax Aa 2.2. Cells bearing gyrB were resistant to novobiocin, indicating that the Haloferax gene is functional in H. salinarium. Complementation of this deletion strain with sopl **fused** to the bacterio-opsin promoter resulted in the recovery of all phenotypical attributes of SRI. This establishes the first direct correlation between sopl and the function of its gene product. In the complemented deletion strain, functional expression of sopl occurred from the bop locus, where sopl had integrated by homologous recombination. This shows that cotranscription of sopl and the gene encoding the SRI signal transducer, htrl, which is found in the wild type, is not a prerequisite for photosensory activity. Deletion of the last 43 bp at the 3' end of sopl resulted in a 10-fold increase in the amount of SRI, without affecting the activity of the pigment. The mRNA level of the truncated gene was not affected as compared to that of the wild type. We propose that regulation occurs at the protein level, probably through a negative determinant of protein stability located in the C-terminus of SRI. Replacement of the last 28 amino acids of **bacteriorhodopsin** by the last 29 amino acids of SRI results in a decrease of the **bacteriorhodopsin**, supporting our observations. The C-terminus of SRI is the first domain with a downregulating influence on protein levels thus far identified in H. salinarium. The system for SRI overexpression we present here greatly facilitates biochemical and biophysical studies on the photoreceptor and allows investigation of the molecular interactions underlying the signal transduction chain of halobacterial phototaxis.

L8 ANSWER 52 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1993:287163 BIOSIS
DOCUMENT NUMBER: PREV199345005288
TITLE: Characterization of chimaeric bacteriorhodopsins produced in Schizosaccharomyces pombe.
AUTHOR(S): Teufel, Michael; Fritz, Hans-Joachim
CORPORATE SOURCE: Inst. Mol. Genetik, Georg-August-Univ. Goettingen, Grisebachstr. 8, W-3400 Goettingen Germany
SOURCE: Protein Engineering, (1993) Vol. 6, No. SUPPL., pp. 69.
Meeting Info.: Winter Symposium on Advances in Gene Technology: Protein Engineering and Beyond Miami, Florida, USA 1993

ISSN 0269-2139.
DOCUMENT TYPE: Conference
LANGUAGE: English

L8 ANSWER 53 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1993:44734 BIOSIS
DOCUMENT NUMBER: PREV199344021584
TITLE: Membrane design starting from **bacteriorhodopsin**.
AUTHOR(S): Teufel, Michael (1); Pompejus, Markus (1); Friedrich, Karlheinz; Fritz, Hans-Joachim (1)
CORPORATE SOURCE: (1) Institut fuer Molekulare Genetik der Universitaet Goettingen, Grisebachstrasse 8, W-3400 Goettingen Germany
SOURCE: Biological Chemistry Hoppe-Seyler, (1992) Vol. 373, No. 9, pp. 867.
Meeting Info.: Autumn Meeting of the Gesellschaft fuer Biologische Chemie (German Society for Biological Chemistry), Rostock, Germany, September 24-26, 1992. BIOL CHEM HOPPE-SEYLER
ISSN: 0177-3593.
DOCUMENT TYPE: Conference
LANGUAGE: English

L8 ANSWER 54 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1992:414753 BIOSIS
DOCUMENT NUMBER: BA94:77953
TITLE: **BACTERIORHODOPSIN** CAN BE REFOLDED FROM TWO INDEPENDENTLY STABLE TRANSMEMBRANE HELICES AND THE COMPLEMENTARY FIVE-HELIX FRAGMENT.
AUTHOR(S): KAHN T W; ENGELMAN D M
CORPORATE SOURCE: DEP. MOLECULAR BIOPHYSICS BIOCHEM., YALE UNIV., NEW HAVEN, CONN. 06511.
SOURCE: BIOCHEMISTRY, (1992) 31 (26), 6144-6151.
CODEN: BICHAW. ISSN: 0006-2960.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB This paper describes experimental tests of the hypothesis that **bacteriorhodopsin** (BR) can fold by the association of independently stable transmembrane helices. Peptides containing the first and second helical segments of BR were chemically synthesized. These two peptides and the complementary five-helix fragment of BR were reconstituted in three separate populations of native-lipid vesicles which were then mixed and **fused** to allow the fragments to interact. After addition of retinal, absorption spectroscopy of the reconstituted BR and X-ray diffraction of two-dimensional crystals of this material showed that the native structure of BR was regenerated. The first two helices of BR can therefore be considered as independent folding domains, and covalent connections in the loops connecting the helices to each other and to the rest of the molecule are not essential for the appropriate association of the helices.

L8 ANSWER 55 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1991:218060 BIOSIS
DOCUMENT NUMBER: BR40:103895
TITLE: CRYSTALLIZATION OF AN ESCHERICHIA-COLI EXPRESSED BACTERIOPSIN **FUSION** PROTEIN.
AUTHOR(S): MITRA A K; MIERCKE L J W; BETLACH M C; SHAND R F; STROUD R M
CORPORATE SOURCE: DEP. BIOCHEMISTRY AND BIOPHYSICS, UNIV. CALIF., SAN FRANCISCO, CALIF. 94143-0448.
SOURCE: THIRTY-FIFTH ANNUAL MEETING OF THE BIOPHYSICAL SOCIETY, SAN FRANCISCO, CALIFORNIA, USA, FEBRUARY 24-28, 1991. BIOPHYS J, (1991) 59 (2 PART 2), 328A.
CODEN: BIOJAU. ISSN: 0006-3495.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L8 ANSWER 56 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1986:181505 BIOSIS
DOCUMENT NUMBER: BR30:93377
TITLE: CALCIUM TRANSPORT IN MEMBRANE VESICLES OF STREPTOCOCCUS-CREMORIS **FUSED** WITH **BACTERIORHODOPSIN** PROTEOLIPOSOMES.
AUTHOR(S): DRIESSEN A J M; HELLINGWERF K J; KONINGS W N
CORPORATE SOURCE: DEP. MICROBIOL., UNIV. GRONINGEN, KERKLAAN 30, 9751 NN HAREN, THE NETH.
SOURCE: SCHAEFER, G. (ED.). ICSU (INTERNATIONAL COUNCIL OF SCIENTIFIC UNIONS) SHORT REPORTS, VOL. 3. THIRD EUROPEAN BIOENERGETICS CONFERENCE; HANNOVER, WEST GERMANY, SEPT. 2-7, 1984. XLVIII+745P. CAMBRIDGE UNIVERSITY PRESS: NEW YORK, N.Y., USA; CAMBRIDGE, ENGLAND. ILLUS, (1985) 0 (0), 405.
CODEN: ISREEB. ISBN: 0-521-30813-5.
FILE SEGMENT: BR; OLD
LANGUAGE: English

L8 ANSWER 57 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1986:158802 BIOSIS
DOCUMENT NUMBER: BA81:69218
TITLE: **FUSION** OF **BACTERIORHODOPSIN** WITH SUBMITOCHONDRIAL PARTICLES YIELDS A NEW SYSTEM WITH RETENTION OF ENERGY COUPLING AND ACQUISITION OF PHOTOPHOSPHORYLATION ACTIVITY.
AUTHOR(S): SEREN S; CASADIO R; SORGATO M C
CORPORATE SOURCE: ISTITUTO DI CHIMICA BIOL. E CENTRO STUDIO FISIOL. MITOCONDRIALE, C.N.R., VIA F. MARZOLO, 3, 35131 PADOVA, ITALY.
SOURCE: BIOCHIM BIOPHYS ACTA, (**1985 (RECD 1986)**) 810 (3), 370-376.
CODEN: BBACAQ. ISSN: 0006-3002.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Submitochondrial particles were fused with purple membranes of Halobacterium halobium cells by means of a freeze-thaw sonication procedure. It is reported that **fusion** of inner mitochondrial membranes with a bacterial membrane yields a new particle which shows not only retention of redox- and photon-linked energy-coupling activities, but also creation of an additional energy-coupling process, light-driven ATP synthesis.

L8 ANSWER 58 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1985:410517 BIOSIS
DOCUMENT NUMBER: BA80:80509
TITLE: LIGHT-INDUCED GENERATION OF A PROTONMOTIVE FORCE AND CALCIUM-TRANSPORT IN MEMBRANE VESICLES OF STREPTOCOCCUS-CREMORIS FUSED WITH **BACTERIORHODOPSIN** PROTEOLIPOSOMES.
AUTHOR(S): DRIESSEN A J M; HELLINGWERF K J; KONINGS W N
CORPORATE SOURCE: DEP. MICROBIOL., UNIV. GRONINGEN, KERKLAAN 30, 9751 NN HAREN, NETH.
SOURCE: BIOCHIM BIOPHYS ACTA, (1985) 808 (1), 1-12.
CODEN: BBACAQ. ISSN: 0006-3002.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The light-driven primary proton pump bacteriorhosopsin was incorporated in the cytoplasmic membrane of S. cremoris, in order to generate a protonmotive force across these membranes. This was achieved by **fusion** of S. cremoris membrane vesicles with **bacteriorhodopsin** proteoliposomes. This **fusion** occurred when both preparations were mixed at low pH (< 6.0), as shown by sucrose density gradient centrifugation and by dilution of fluorescent phospholipids incorporated into the **bacteriorhodopsin** proteoliposomes. **Fusion** was strongly enhanced by the presence of

negatively charged phospholipids in the liposomal bilayer. When proteoliposomes were used that showed light-dependent proton uptake, the orientation of **bacteriorhodopsin** in the fused membranes was inside-out with respect to the in vivo orientation in Halobacterium halobium. In the light a transmembrane electrical potential, interior positive and a .DELTA.pH, interior acid were generated. This protonmotive force could drive Ca uptake in the fused membranes. The uptake increased hyperbolically with increasing light intensity and was abolished by bleaching of **bacteriorhodopsin**. Addition of the ionophore valinomycin stimulated Ca uptake and led to an increase of the .DELTA.pH. Ca uptake was strongly decreased in the dark and in the light in the presence of uncouplers, nigericin or both valinomycin and nigericin.

L8 ANSWER 59 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1985:149802 BIOSIS
DOCUMENT NUMBER: BR29:39798
TITLE: BIOLOGICAL MEMBRANES VOL. 5.
AUTHOR(S): CHAPMAN D
CORPORATE SOURCE: DEP. OF BIOCHEMISTRY AND CHEMISTRY, ROYAL FREE HOSPITAL SCHOOL OF MEDICINE, UNIVERSITY OF LONDON, ENGLAND.
SOURCE: CHAPMAN, D. (ED.). BIOLOGICAL MEMBRANES, VOL. 5. XIV+494P. ACADEMIC PRESS INC., PUBLISHERS: ORLANDO, FLA., USA.
ILLUS,
(1984 (RECD 1985)) 0 (0), XIV+494P.
CODEN: BMEMEL. ISBN: 0-12-168546-2.
DOCUMENT TYPE: Book
FILE SEGMENT: BR; OLD
LANGUAGE: English
AB This updated volume provides current works in the field of biomembrane research. The book is comprised of 11 chapters. Chapter 1 discusses the concept of biomembrane fluidity. The topic of how biomembranes may be stabilized while experiencing dehydration is discussed in chapter 2. The triggering processes and membrane structures are indicated in chapter 3. Rhodopsin's role in visual transduction, **bacteriorhodopsin** topography in purple membrane, and the function of the acetylcholine receptor structure is assessed in chapters 4, 5 and 6, respectively. Chapter 7 examines two methods for researching fast trigger processes and chapter 8 covers conformation changes in membrane proteins. Chapters 9 and
10 deal with calcium and cellular activation and the ordered water model of membrane ion channels. The new technique of electric field induced **fusion** of cell biomembranes is described in the final chapter. This treatise is supplied with graphs, photomicrographs, figures, references, and author and subject indices.

L8 ANSWER 60 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1983:295851 BIOSIS
DOCUMENT NUMBER: BA76:53343
TITLE: MONO CLONAL ANTIBODIES TO RHOD OPSIN CHARACTERIZATION CROSS
REACTIVITY AND APPLICATION AS STRUCTURAL PROBES.
AUTHOR(S): MOLDAY R S; MACKENZIE D
CORPORATE SOURCE: DEP. OF BIOCHEMISTRY, UNIV. OF BRITISH COLUMBIA, VANCOUVER,
BRITISH COLUMBIA, CAN. V6T 1W5.
SOURCE: BIOCHEMISTRY, (1983) 22 (3), 653-660.
CODEN: BICHAW. ISSN: 0006-2960.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Two monoclonal antibodies designated as rhodopsin (rho) 1D4 and rho 4A2 were obtained from hybridoma cells cloned after the **fusion** of mouse myeloma cells with spleen cells of a mouse immunized with bleached bovine rod outer segment disk membranes. These antibodies were specific for rhodopsin as determined by radioimmune labeling of bovine rod outer segment disk membrane proteins electrophoretically transferred from sodium
dodecyl sulfate gels to CNBr[cyanogen bromide]-activated paper. Limited proteolytic digestion of rhodopsin in sealed disk membranes in conjunction

with radioimmune assays indicated that the rho 1D4 antibody bound to the carboxyl-terminal segment of rhodopsin on the cytoplasmic side of disk membranes, whereas the rho 4A2 antibody bound to a determinant along the amino-terminal third of the rhodopsin polypeptide chain. Binding of the rho 4A2 antibody was sensitive to solubilization and photobleaching of rhodopsin. The rho 4A2 antibody did not bind to rhodopsin in sealed membrane disks but did bind to detergent-solubilized rhodopsin. Detergent-solubilized bleached rhodopsin was 13 times more antigenic than unbleached rhodopsin. Rhodopsin solubilized in Triton X-100 was more antigenic than rhodopsin solubilized in cholate. Apparently, the 4A2 antibody serves as a sensitive immunological probe for structural changes of rhodopsin caused by solubilization and photobleaching. Both the rho 1D4 and 4A2 antibodies were also found to cross-react with frog rhodopsin but not H. halobium **bacteriorhodopsin**. The rho 4A2 antibody bound to the 3 forms of frog rhodopsin resolved by sodium dodecyl sulfate gel electrophoresis; rho 1D4 bound to only the 2 higher MW frog rhodopsins. Lectin inhibition studies using 125I-labeled succinyl-Con A [concanavalin A] and antibody inhibition showed freshly prepared bovine disks were sealed with the lectin binding sites oriented toward the inside of the disk; frozen-thawed disks were predominantly unsealed with both membrane surfaces exposed. Frog disk membrane vesicles were shown to have the same orientation.

L8 ANSWER 61 OF 61 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1980:219562 BIOSIS
DOCUMENT NUMBER: BA70:12058
TITLE: HALOBACTERIUM-HALOBIUM 1. IN-VITRO STUDIES.
AUTHOR(S): HIGGINS J; LOPEZ J R; TIEN H T
CORPORATE SOURCE: DEP. BIOPHYS., MICH. STATE UNIV., EAST LANSING, MICH. 48824, USA.
SOURCE: BIOELECTROCHEM BIOENERG, (1979 (RECD 1980)) 6 (4), 509-524.
CODEN: BEBEBP. ISSN: 0302-4598.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Purple membrane fragments (PM) from H. halobium were incorporated into lipid membranes either directly or via liposomes. A photoresponse was detected when liposomes containing PM were **fused** with lipid membranes. Low-resistance membranes or membranes shunted with an external resistor of 109 ohms showed decay of the initial light response to some equilibrium value in the light in both presence and absence of octadecylamine. The light response could be abolished by the addition of a sufficient amount of triethylamine to either side of the membrane. The photovoltage action spectra of bilayer lipid membranes containing PM either directly or via liposomes were measured, and they followed the absorption spectrum of **bacteriorhodopsin**. Liposomes containing **bacteriorhodopsin** (BR) in PM extract of H. halobium were **fused** to 1 side of planar lipid membranes. The photopotential resulting from flash excitation rose and fell as the sum of 3 exponentials with time constants for the leading edge of 30 .+-. 10 .mu.s and 35 .+-. 10 ms. The decay time constant of the photopotential was 840 ms, a value consistent with the membrane time constant given by the membrane dark resistance and capacitance.

=> log y